H I L G A R D I A

A Journal of Agricultural Science Published by the California Agricultural Experiment Station

VOL. 29 JANUARY, 1960 No. 10

FACTORS AFFECTING EGG PRODUCTION AND OVIPOSITION IN POPULATIONS OF *COLIAS PHILODICE EURYTHEME* BOISDUVAL (LEPIDOPTERA: PIERIDAE)[1]

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INTRODUCTION

THE ALFALFA CATERPILLAR, *Colias philodice eurytheme* Boisduval, is a native insect described from California about four years before the introduction of alfalfa (Boisduval, 1852; Hendry, 1925).[4] Originally, its distribution was restricted to the western portion of North America, but with removal of the forest and grass cover and the planting of alfalfa in other areas, the geographical distribution was greatly extended (Hovanitz, 1944, 1945; Gerould, 1946; Smith and Allen, 1954). At present, *Colias* is found from the Atlantic to the Pacific oceans and from southern Canada to southern Mexico (Hovanitz, 1944, 1950).

Before the introduction of alfalfa into California, the alfalfa caterpillar probably occurred in low numbers over much of the state on a variety of native legumes. Such common species as *Lupinus succulentus* Douglas, *Lupinus bicolor* Lindley, *Trifolium tridentatum* Lindley, and *Lotus subpinnatus* Lagasca were undoubtedly important hosts and, to a lesser extent, other species in these genera and in *Astragalus, Vicia, Lathyrus,* and *Psoralea* contributed to the food supply. The greatest numbers of caterpillars probably developed on a seasonal succession of legumes during wet springs. In the hot, dry summers, the quantity of suitable host plants was reduced, but a few caterpillars developed in favorable spots in moist river bottoms. With the cultivation of irrigated alfalfa, a new and abundant source of food was provided for both larvae and adults. As a result, not only was the geographical distribution of the species extended, but the average density was also raised. Furthermore, in contrast to the earlier conditions, the peak population levels now occur during the hot, dry summer (Michelbacher and Smith, 1943). In other words, introduction of alfalfa and irrigation has

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[4] See "Literature Cited" for citations referred to in the text by author and date.

modified the ecosystem of *Colias philodice eurytheme,* resulting in a higher general equilibrium position. The California climate, the inherent characteristics of *Colias,* and the mortality factors of the environment have not changed appreciably in quality. The changes have been brought about by alterations in quality and quantity of food supply, and modification of local climates and microenvironments produced by cultivation of alfalfa.

From the viewpoint of local population dynamics (the viewpoint of the alfalfa grower), the factors causing variations from season to season and site to site are of primary interest. Such local variations occurred before the introduction of alfalfa, and now occur from one alfalfa field to another as well as within a single field. The level of these local infestations depends on the number of eggs laid on the alfalfa and the subsequent development and mortality of the eggs and larvae. The mortality factors, which are variable, have been discussed elsewhere (Michelbacher and Smith, 1943; Smith and Allen, 1954; Allen, 1958; Allen and Smith, 1958; Thompson and Steinhaus, 1950).

The primary objective of the present study, conducted from 1949 to 1952, was to determine some of the factors influencing egg production and oviposition of *Colias philodice eurytheme,* and their significance in population fluctuation.

The study was approached from three different aspects. First, those factors such as the morphology of the reproductive system, mating habits, and oögenesis were considered since they are a prelude to oviposition. Second, the characteristics of the environment, especially the physical factors, were investigated since they are important in influencing the oviposition rates. Finally, population phenomena apart from the activities of the individual and the effects of the environment were considered in relation to oviposition.

GENERAL BIOLOGY

Colias philodice eurytheme lays eggs singly on shoots or leaves of young alfalfa. In the hot summer, the eggs hatch in three days or less, while in cooler periods the incubation period may be greatly extended. At 25° C, the eggs hatch in about four days; the larval period requires about 17 days, and the pupal period about seven days (Allen and Smith, 1958). The small larvae eat very little, and remain near the oviposition site. The greatest consumption of food occurs during the fourth and fifth instar. The fifth instar larva will eat a dozen or more leaves a day. When mature, the larva usually moves from the top of the plant to pupate nearer the ground (Michelbacher and Smith, 1943; Smith, 1949). The females normally mate in the field from which they emerged, and then disperse. Because the female prefers to oviposit on young alfalfa growth, recently-cut alfalfa fields supply abundant oviposition sites. Females found ovipositing in such fields have usually emerged in another field in the vicinity (Smith, Bryan, and Allen, 1949).

In the San Joaquin Valley, larvae survive the winter in the semidormant alfalfa fields, while in southern California, all life stages can be found in winter. In the former area, most of the overwintering brood usually emerges in March. The renewal of larval development in the spring coincides with

growth of the alfalfa, and most larvae have pupated and emerged before the first spring cutting (Smith, 1949; Smith and Allen, 1954; Allen and Smith, 1958).

A significant preadaptation of *Colias philodice eurytheme* to alfalfa culture is the preference of the females to oviposit on short, succulent alfalfa shoots. This produces even broods which can complete their development within a single alfalfa cutting period. For example, in the second cutting period, the alfalfa starts its growth more or less parallel to the development of first eggs laid on it by the overwintering brood. Usually oviposition continues on the young alfalfa until it is about one-third grown. When the alfalfa is cut, only the larvae that have completed or nearly completed their development will survive. The emerging females mate and disperse to oviposition-stage fields to begin the cycle over again. The oviposition habits of the adults are thus of major significance in development of economic populations of larvae such as occur periodically in California, Arizona, and other parts of the West.

The synchronization of *Colias* with the cutting periods continues throughout the summer and into the fall. In autumn, cool weather retards the growth of both alfalfa and the *Colias* larvae. The adults of the late fifth and sixth broods still oviposit on the slowly-growing alfalfa until cold weather eliminates them (Allen and Smith, 1958).

MATING AND OÖGENESIS

Female Reproductive System

The gross anatomy of the female reproductive system of *Colias philodice eurytheme* (fig. 1) does not differ greatly from that of other Lepidoptera with two genital apertures (Jackson, 1889; Eidmann, 1929*a*; Bourgogne, 1951; Imms, 1957). The internal and external female reproductive organs of *Colias* have been briefly described by Gerould (1925). The terminology used in the following description of the female reproductive system has been modified from Kusnezov (1915), Norris (1932), and Mariani (1937).

The copulatory aperture (ostium bursae) is ventral to the ovipositor, and appears as a strongly-chitinized, brownish, oval opening.

The bursa copulatrix can be divided into three general structures, the ductus bursae, corpus bursae, and appendix bursae. The ductus bursae is inward from the copulatory aperture and somewhat parallel to the long axis of the body. It is immediately compressed directly to the interior, into a thin, flattened, strongly-chitinized structure curved downward slightly. Halfway between the copulatory aperture and the juncture of the ductus seminalis, the ductus bursae is slightly evaginated. At this point, a slight lobe appears dorsally on the evagination, and the ductus bursae widens and starts to curve upward.

At the juncture of the ductus seminalis, the ductus bursae loses its flattened appearance. This portion, the cervix bursae, assumes different shapes, depending on whether or not the female has mated. In the unmated female, the cervix bursae is a round, knobby, gnarled structure opening into the uninflated corpus bursae. In the mated female, the cervix bursae widens in

a conelike manner, and suddenly flares into the inflated, round corpus bursae. The corpus bursae of *Colias philodice eurytheme* consists of a shriveled sac in the unmated female (fig. 3A, p. 423), while in the successfully mated female it takes the shape of the spermatophore (fig. 3B and C, p. 423). The latter is usually spherical, somewhat flattened at the top, and contains a neck at the bottom. The size of the corpus bursae will vary, depending on the size of the spermatophore and the number of times the female has been successfully

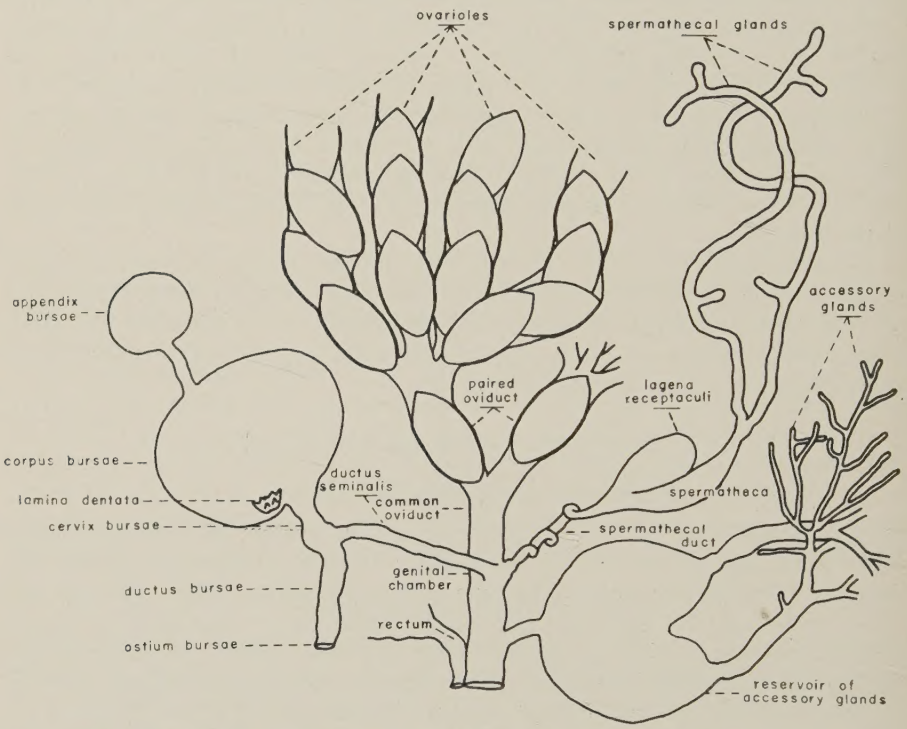

Fig. 1. Reproductive system of a mated female *Colias philodice eurytheme* with mature eggs.

mated. A female may mate as many as four times. A successful mating is herein considered to be the passing of a spermatophore.

The spermatophore, which is formed in the female, is produced from the glandular secretions of the male reproductive ducts, and conveys the spermatozoa into the corpus bursae of the female. Since the spermatophore is not a structure of the female reproductive system, it is discussed in more detail under "Mating" (p. 419).

An interesting feature of the corpus bursae is the lamina dentata (Hagen, 1882; Stitz, 1901). Its size and shape vary quite extensively from one group of Lepidoptera to another. In some, it is a large, chitinized, toothed structure, while in others very small teeth are present (Bourgogne, 1951). When present, the lamina dentata may offer specific morphological characters (Jackson, 1889; Petersen, 1904; Mariani, 1937). In the genus *Colias*, it has

been used with other characters to distinguish the closely related *Colias electo* (Linnaeus), *C. croceus* (Fourcroy), *C. pseudohecate* (Berger), and *C. fieldii* (Ménétriés) (Lempke, 1946; Jarvis, 1953), and also *C. australis* (Verity) and *C. hyale* (Linnaeus) (Jarvis, 1955).

In *Colias philodice eurytheme,* the lamina dentata is situated in the corpus bursae just as it begins to flare from the cervix bursae. Its large teeth or hooked spines face away from the cervix bursae and toward the outside of the corpus bursae, while the smaller teeth face the opening of the cervix bursae. From above, a groove can be seen separating the lamina dentata into two more or less asymmetrical parts. The teeth of the lamina dentata point away from this groove. The tips of the lamina dentata point dorsally. In this species, the size of the lamina dentata varies, but usually it is about 1 mm in length and about 0.25 mm at its widest point (fig. 3, p. 423).

The function of the lamina dentata has not been clearly demonstrated. Petersen (1904) thought its purpose might be to break the spermatophore and thus allow the spermatozoa to escape. Norris (1932), however, found that an opening already exists in the spermatophore of *Anagasta kühniella* (Zeller) and *Plodia interpunctella* (Hübner) and that this opening appears to be the exit. The same is true for *C. philodice eurytheme.* Mariani (1937) states that the spermatophore is enveloped within a thin cuticle, and that it is the function of the lamina dentata to break this cuticle in order to allow the spermatozoa to pass from the corpus bursae to the ductus seminalis.

The appendix bursae is connected to the top of the corpus bursae by a slender duct. In the unmated *C. philodice eurytheme* female, it is an uninflated sac similar to the corpus bursae. Immediately after mating, the structure fills with a white secretion, becomes inflated, and assumes a round shape much the same as that of the corpus bursae. It lies above and in front of the corpus bursae, directly beneath the abdominal tergites. The four coiled ovarioles are immediately ahead of the corpus bursae and appendix bursae on each side of the body. Some reports have indicated the presence of spermatozoa in the appendix bursae in other species; however, none was found in appendix bursae of *C. philodice eurytheme.*

The ductus seminalis is a narrow duct which serves as a passage for the spermatozoa from the spermatophore to the genital chamber where they then enter the spermathecal duct. The ductus seminalis joins the ductus bursae at the point where the ductus bursae loses its flattened appearance and assumes a funnel shape. It is not a straight tubule, but curves in various directions until it joins the genital chamber. In some Lepidoptera, another structure, the bulla seminalis, is found along the ductus seminalis, but this is not present in *C. philodice eurytheme.*

Embryonated eggs were occasionally found in the ductus seminalis, as Gerould (1925) has also observed. In many specimens, embryonated eggs were found in the lower portion of the common oviduct. The development of the embryonated eggs in the female has been noted for other *Colias* (Kusnezov, 1909; Harbottle, 1950; Frazer, 1951). Often when females are killed, but not dissected immediately, the eggs continue to develop, and in some cases small larvae may be found in the female. Pierce (1911) found such a larva in *Colias croceus,* and Klots (1935) found one in the oviduct

of *C. hecla* Lefebre. Kusnezov (1909) observed larvae in the adult female in 23 species of pierids, of which 16 were *Colias* species. These authors have referred to such larvae as a possible indication of ovoviviparity. Most of the specimens examined by Kusnezov were northern or high-altitude *Colias* species, from which he concluded that ovoviviparity may be considered to be climatically controlled since it would be an expedient adaptation to the short period of vegetation in northern or elevated countries. Kusnezov further suggested that an individual may alternate normal oviposition with ovoviviparity. It is assumed that the females he studied had been dead for some time, because he softened his specimens in 10 per cent potassium hydroxide solution. He states that boiling the specimens in potassium hydroxide does not destroy the chorion; however, in the present study the chorion was destroyed if the specimens were held too long in the boiling solution. On the other hand, if the specimens were held in the solution for a very short time, and the body contents were teased out, the larvae, if present, could be found within the chorion.

In this study, adult female *Colias philodice eurytheme* were first killed by crushing the thorax, and then held at 25° C. After one day, none of the females dissected had any embryonated eggs as judged on the basis of color. (In this species, fertilized eggs turn from white, to pink, to red, and then to black. After the egg turns black, the larva can be seen inside.) After two days, three out of 13 females dissected had a pink egg in the lower oviduct. On the third day, red eggs were found, and on the fourth day the larvae could be distinguished. In one specimen, the egg with larva was found in the ductus seminalis. It was not determined whether the larvae could emerge from dead females.

Random sampling of museum specimens of *Colias* from several California localities also indicated the presence of larvae in the females. Five out of 10 females from Berkeley, three out of 12 from Westley, three out of nine from Ridgecrest, and none out of 21 from Blythe contained larvae. These specimens had been dead for two to five years.

In areas of low host density, or when conditions are not favorable for oviposition, the female may be unable to deposit fertilized eggs immediately. As she seeks suitable hosts or waits for favorable oviposition conditions, the eggs may continue their development. From our observations, it is extremely unlikely that live females would larviposit or lay eggs containing unhatched larvae. Oviposition pressure ordinarily would force them to oviposit before such a stage was reached. On the basis of the evidence given here, the mere presence of larvae in dead females cannot be taken as proof of ovoviviparity (Hagan, 1951). If the adult were killed, she would not be capable of peristaltic movement. Therefore eggs might continue to develop inside her body —something which they would not do normally.

The crescentic spermatheca (receptaculum seminis) is connected to the genital chamber by the thin, helical spermathecal duct (ductus receptaculi). It is located under the accessory glands and the cervix bursae, and is attached to the cervix bursae by tracheae and other tissues. In a fertilized female, a mass of white sperm can be seen inside the spermatheca, while in the unfertilized female this structure is translucent.

Adjoining the spermatheca is the lagena receptaculi, which resembles a hanging drop of water. After mating has occurred, this structure may or may not contain a white solution similar in appearance to that found in the appendix bursae. In mated females, the white material is always found in the appendix bursae, whereas it is found only occasionally in the lagena receptaculi. Although a large number of dissections were made, spermatozoa were found in lagena receptaculi only once. The spermatheca extends into a thin tubule which later branches into the two spermathecal glands (glandula receptaculi), which wind around the corpus bursae. Short tubes branch from these two glands at two or three positions. The number and position of these branches vary from individual to individual. Norris (1932) suggests that the secretion of these glands is probably a nutritive medium for the spermatozoa.

In the young, mature female, the ovarioles, four on each side of the body, occupy most of the abdomen, while in newly-emerged and old adults, the ovarioles are much smaller. Eggs of all sizes are found in the mature adult. In some cases, the mature eggs may overlap to a considerable degree. If the female has had no chance to oviposit, as many as three to four mature eggs may be clustered together in the position normally occupied by a single egg in a freely ovipositing female. In an old adult that has oviposited freely, the ovarioles and their eggs have nearly disappeared, whereas in the newly-emerged adult the eggs are immature, and thus occupy little space within the body (Eidmann, 1929*b*). The ovarioles coil dorsally a number of times, depending on how many eggs have been laid. In the newly-emerged and mature females, the ovarioles are bound in a compact mass of fat body and tracheae; in the old adult, the fat body is completely depleted.

In the newly-emerged female, no eggs appear in the paired or common oviduct. At this stage the eggs are about one-sixth their final size, and as a single ovariole is traced toward the germarium, the eggs become smaller and smaller. It is quite easy to count over 125 such eggs in each ovariole of most newly-emerged females. This would be equivalent to more than 1,000 eggs visible in the average young adult. If conditions are favorable, and a female is allowed to oviposit freely, very few mature eggs will be found in the body since a female will tend to deposit her eggs as soon as they mature. If a female is held in the dark, she will not oviposit freely, and the ovarioles soon become packed with eggs. Unfertilized females appear to oviposit only when unable to retain the developing eggs.

In the newly-emerged female, the bilobed reservoir of the accessory glands is quite small and translucent, while in the older adult the reservoir is greatly enlarged and filled with a milky white fluid. The reservoir of the accessory glands joins the genital chamber at nearly the same position as does the rectum. The two lobes of the accessory gland reservoir fit one on each side of the cervix and corpus bursae. At the tips of each lobe, the accessory glands extend out and branch considerably. They are compressed into a small space, one on each side, at the tip of the abdomen. The secretion from the accessory gland apparently serves to attach the eggs to the plant (Norris, 1932; Mariani, 1937).

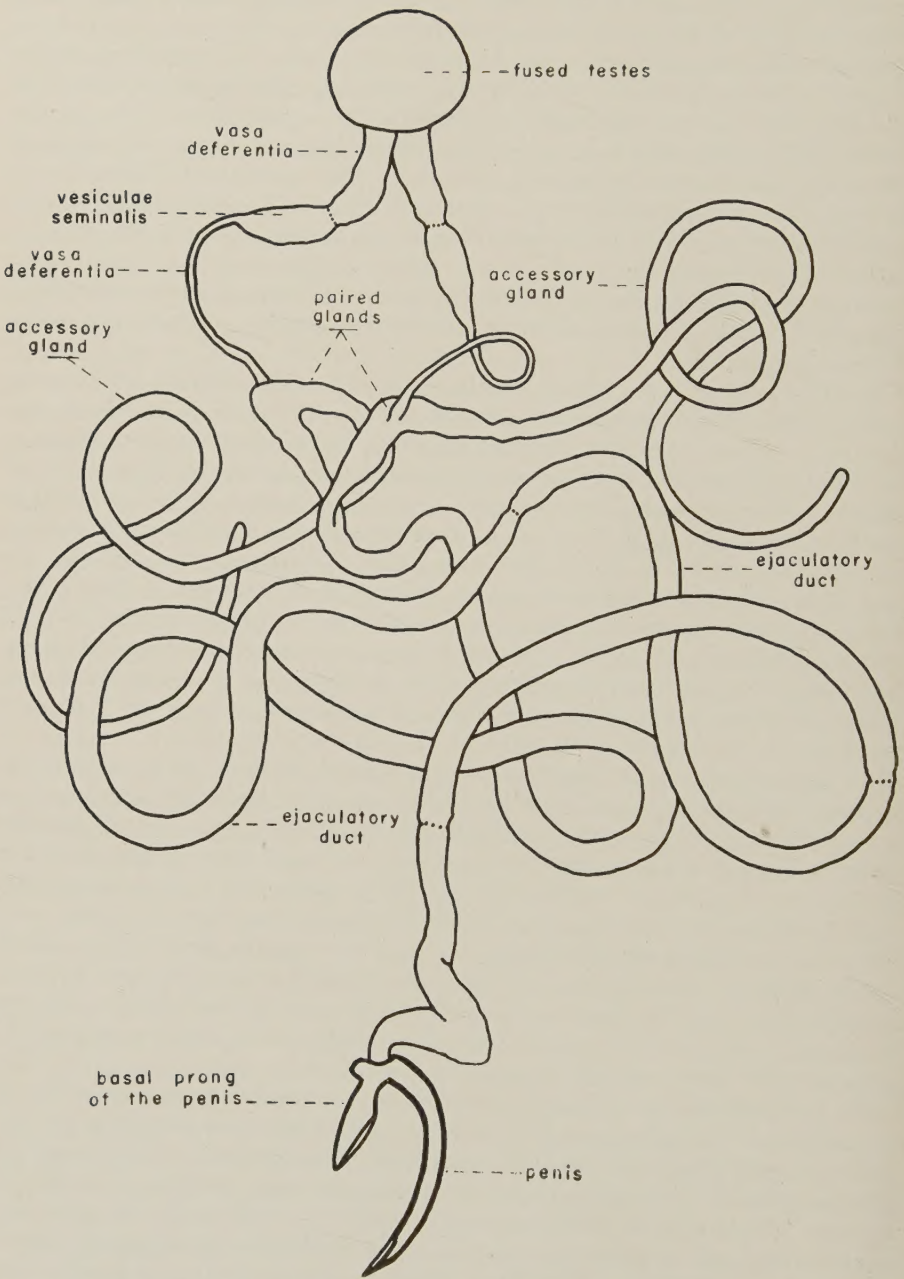

Fig. 2. Reproductive system of male *Colias philodice eurytheme*.

Male Reproductive System

The male reproductive system of *Colias philodice eurytheme* (fig. 2) is typical of the majority of Lepidoptera in which the testes are fused (Imms, 1957; Cholodkowsky, 1884; Bourgogne, 1951; Snodgrass, 1935), and is not unlike that of *Pieris* (Mehta, 1933). The terminology used here has been modified from the work of Hewer (1932), Norris (1932), Klots (1935), Imms (1957), and Musgrave (1937).

The testes in the newly-emerged male lie just beneath the tergites. The two testes are spirally wound about one another, and each twisted lobe is composed of four follicles. Within these follicles can be found the mature bundles of spermatozoa (Gerould, 1925). They are imbedded ventrally in a mass of fat body, but above the testes, fat tissue is scattered. The testes are easily recognized by their reddish-white color. Their outer surface seems to be covered by scattered deposits of whitish tissue which partially hide their darker red appearance. If the testes are dissected, an even greater amount of red coloration is to be found along the longitudinal axis.

The upper portions of the vasa deferentia are enlarged in this species. A slight constriction in each tubule a short distance below the testes delimits the upper end of the vesiculae seminalis. Below the vesiculae seminalis, the vasa deferentia are narrow tubules. At their lower ends, they join the paired glands and are surrounded by the accessory glands.

The paired glands are large and U-shaped. The accessory glands join the paired glands and are narrow tubules which attenuate toward their tips. The two accessory glands are intermittently joined by connective tissue and tracheae, so that they lie adjacent for most of their length. Toward their tips they cross over one another, but do not interwind.

The ejaculatory duct is three to four times the length of the abdomen. It is divided into four sections, three long and one very short. Each section is separated by a constriction. Norris (1932) divided this duct into four sections in *Plodia* and *Anagasta* on the basis of their secretions. On the basis of macroscopic examination, the duct and its contents also appear to be different in each of the sections in *Colias philodice eurytheme*. The ejaculatory duct is enclosed in white, muscular tissue at its distal end. This structure may be comparable with what Norris (1932) refers to as the bulbus ejaculatorius. It joins the hard, brown, heavily chitinized penis abruptly, so that there is a definite line of demarcation between the penis and the lower portion of the ejaculatory duct.

Mating

As with other species of butterflies (Tinbergen *et al.*, 1942; Newman, 1949), *Colias* appears to follow a definite precopulatory or nuptial pattern of activity comprised of special flights, and wing and abdominal movements while at rest. The males tend to emerge before the females, and their wings are always fully hardened before copulation. The wings of the female are not always hardened before copulation, and in some cases, they may not be fully expanded. On the other hand, field-collected females show that generally they do not mate until their wings have dried, even though abundant males

are present. The precopulatory wing movements in soft, freshly-emerged females are limited in comparison with the wing movements in older females. In either case, the male flutters about the female and then settles near her. The fully hardened female flutters her wings, occasionally holding them horizontally, and lifts the tip of the abdomen. As she does this, she forces the chitinous plate surrounding the ovipositor and ostium bursae outward, thus fully exposing the external genitalia. The male, meanwhile, if not already facing in the opposite direction, turns and backs toward the female. The female at this time holds her wings vertically and pulls them forward to expose the tip of the abdomen. When the male has moved adjacent to the tip of the female abdomen, he twists his abdomen slightly to the side and lifts it up at a 45-degree angle to bring the tip of the aedeagus above the end of the female abdomen. He then moves his abdomen downward, grasping the chitinous plate surrounding the ovipositor and ostium bursae with his uncus and claspers. The penis then enters the ostium bursae, and the wings of the two sexes interfold. This position and procedure in mating is typical of most butterflies (Richards, 1927). Many mating couples are often seen flying in this position with the male carrying the female. The male also carries the female in *Colias croceus, C. hyale,* and *C. palaeno,* and in other pierines (Donzel, 1837; Turner, 1916; Dixey, 1917).

In alfalfa fields where adults are emerging, females are often seen in what appears to be a flirtation flight. They make short, fluttering flights with males in attendance. The two sexes often touch each other in flight. Usually these flights occur in an area of a few square feet, while at other times the female may fly for some distance with a male or males in chase. In other instances, after fluttering about, the female will fly 100 to 200 feet in the air with the male or males following. In every such case observed, the females always flew higher, thus escaping, while the male or males returned to the ground first. In examining these females after they had returned to the ground, it was found that they contained a spermatophore. The mating of such females must have occurred earlier, since the interlocking of the two sexes cannot be accomplished in flight. Moreover, successful mating could not have occurred in these short flights since the time elapsed would appear to be too short for the passage of the spermatophore.

In oviposition fields, a female may often be seen resting on the alfalfa with males fluttering about her. The female will flutter her wings and occasionally life the tip of the abdomen; then either the attendant males or the female flies away. When such females were dissected, it was found that they contained spermatophores. Thus, while it appears that the fluttering of the wings by the female is part of the precopulation act, it also appears that it can be a method of avoiding the male. A successful mating requires a series of behavioral steps before the spermatophore is passed (Tinbergen *et al.,* 1942). The methods of avoiding the male appear to feign a portion of the precopulatory pattern, but the act is not carried to completion.

Time of Mating. Wildermuth (1914), Gerould (1927), Hovanitz (1949), and others state that mating occurs soon after emergence. This is generally true, but there is some variation. At a very low population density, the male

or female must spend some time seeking the opposite sex. In the winter and spring months, during inclement weather, the adults may not mate for days because the environmental conditions are not suitable for flight and searching.

In the San Joaquin Valley on very hot days, mating as well as general activity is more pronounced during the morning and late afternoon, while during midday, activity nearly ceases. On mild days, mating and other activity occur throughout the day. On cold days, there is very little activity (Hovanitz, 1948; Leigh and Smith, 1959).

In the field, mating appears to depend more upon the response of the female than of the male, while in the laboratory the male appears to lack the necessary stimulus. Usually females that copulated in the laboratory contained no spermatophore. The males appeared to be normal, but under our laboratory conditions, some critical factor necessary to stimulate the passage of a spermatophore was lacking.

Number of Successful Matings. In the present study, a successful mating was considered to be the passage of a spermatophore. In the field, it is easy to determine if a female has been successfully mated by opening the abdomen with the fingers and examining the corpus bursae for the presence of a spermatophore, which is quite large and easily seen with the unaided eye. This method of rapid field observation indicated that virtually all females capable of flying had mated at least once when captured in the field. Laboratory dissections showed that females may mate as many as four times, although this is unusual. This number of matings undoubtedly yields an adequate supply of spermatozoa, but with a spermatophore passed with each mating, their combined size would appear to fill the capacity of the corpus bursae. Perhaps if each spermatophore were very small, a female could mate more than four times.

The average number of matings per female is more than one. The average number of spermatophores in a group of 94 females collected from emergence- and oviposition-stage fields was 1.23. A group of old females taken from an oviposition-stage field was found to have mated 1.60 times. Young females usually have mated but once. These data are summarized in table 10.

No observations were made on the number of times the males can mate. Gerould (1911*a*) stated that a male can often mate on successive days with several different females, and that one male mated five times (Gerould, 1923). However, he does not indicate whether spermatophores were passed. Undoubtedly, small spermatophores are passed if mating occurs too often, since the male must require some time to produce the sperm bundles and the glandular secretion which forms the spermatophore.

Spermatophore. The spermatophore in *C. philodice eurytheme* is a hard, spherical structure, slightly flattened on top, with a long, cone-shaped neck at the bottom. The spherical portion of the spermatophore rests in the corpus bursae, while the neck extends into the cervix bursae as far down as the entrance to the ductus seminalis. There is a small hole at the tip of the neck which lies adjacent to the ductus seminalis, and through this hole the sperm escape. Figure 3 shows the corpus bursae of an unmated, a singly-mated,

and a triply-mated female. The drawings also show some detail of the spermatophore. Occasionally, the general outline of the lamina dentata will be found in the form of a groove in the spermatophore, but in the many females that were dissected, no spermatophore was ever found that had been imbedded in the teeth of the lamina dentata.

The size of the spermatophore varies considerably. One was found to be nearly 2 mm wide and over 2 mm long (including the neck). In another instance, the spermatophore was less than 0.5 mm wide and about 1 mm long. The size of the spermatophore will vary, depending on the amount of glandular solution secreted by the male during copulation. After the male and female have separated, it is usually possible to determine whether a spermatophore has been passed by pinching the tip of the female abdomen. When the spermatophore is very small, the female must be dissected to detect it. On the other hand, when a large spermatophore is passed, it may be noted by the bulging of the abdomen. This is also apparent when the female has mated more than once even though the spermatophores are medium-sized. In dead females, if the spermatophore is large, a bulge usually appears in the shrunken abdomen.

The spermatophore hardens soon after being deposited in the female. If present, it was already a hardened structure in many fresh females caught *in copulo*.

The neck can usually be pried loose from the body of the spermatophore. Spermatophores from earlier matings do not always retain the neck. The last spermatophore from any number of matings always has a neck, which assures the spermatozoa a means of entering the ductus seminalis. In some females the neck of the spermatophore is found within the corpus bursae; this may indicate that the female is preparing to mate again. The sperm exit is oval-shaped, and lies on the side and tip of the neck adjacent to the entrance to the ductus seminalis. Within a short time after mating, the spermatozoa have escaped from the spermatophore and can be seen as a white mass within the spermatheca.

Preoviposition Period

Although females tend to mate soon after emergence, eggs are not laid immediately since the female must pass through a brief preoviposition period (Eidmann, 1929*b*). There are no mature eggs in the ovarioles at the time of eclosion, and the development rate of the eggs determines the preoviposition period.

Normally, the absence of spermatozoa in the spermatheca is not a factor controlling the preoviposition period because the spermatozoa apparently reach the spermatheca before any eggs become mature. However, in some cases, eggs could mature before the female mates.

Twenty-one females from field-collected, copulating pairs were held at 5° C for 19 hours and then at 10° C for 24 hours. Of the 21, nine contained a single spermatophore, one had two spermatophores, and 11 contained none. The eggs of the twice-mated female were mature, and she apparently had oviposited earlier. The spermatheca was full of spermatozoa in the nine females which contained a single spermatophore, and none had mature eggs;

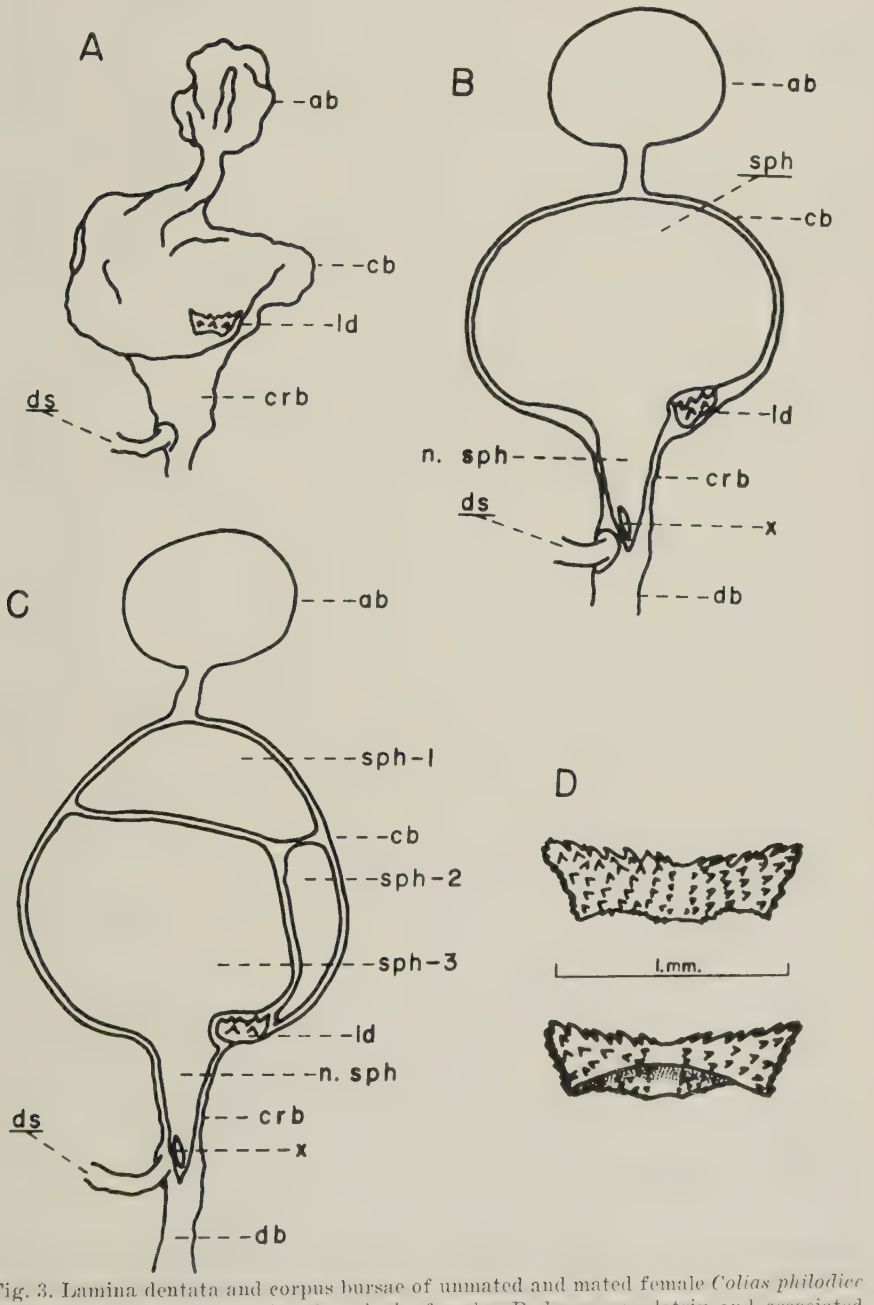

Fig. 3. Lamina dentata and corpus bursae of unmated and mated female *Colias philodice eurytheme*. A. Bursa copulatrix of a virgin female; B. bursa copulatrix and associated structures of a female that has been mated once; C. bursa copulatrix and associated structures of a female that has been mated three times; D. lamina dentata. Lower view partially shows the hollowed interior. Legend: ab—appendix bursae; cb—corpus bursae; crb—cervix bursae; db—ductus bursae; ds—ductus seminalis; ld—lamina dentata; n. sph—neck of spermatophore; sph— spermatophore; sph-1—spermatophore of first mating; sph-2—spermatophore of second mating; sph-3—spermatophore of third mating; x—sperm exit.

the spermatozoa had reached the spermatheca even though the females were held at 10° C and below.

On another occasion, 29 copulating, field-collected females were dissected after being held at 5° C for 19 hours, and then at 25° C for 24 hours. Sixteen females contained a single spermatophore while 13 contained none. Of the 16 containing a spermatophore, 11 had at least one, but not more than 10 mature eggs per ovariole. The remaining five females had no mature eggs. The spermatheca contained spermatozoa in all 16 mated females. Thus, it would appear that if the female mates immediately after emergence, the spermatozoa enter the spermatheca before the eggs mature, even

TABLE 1

MATURATION OF EGGS IN FEMALE *COLIAS PHILODICE EURYTHEME* HELD IN THE DARK AND GIVEN SUGAR-WATER FOR FOOD

Air temperature	Hours from eclosion to dissection	No. of females in sample	No. of females containing mature eggs	Mean no. of eggs per female ± S.E.M.	Standard deviation	Eggs produced per female per hour
°C						
10	570 ± 4	8	1	1 ± 4	1	0.002
15	235 ± 4	21	14	23 ± 5	24	0.1
25	15 ± 4	24	1	0.4 ± 0.4	2	0.03
25	51 ± 4	10	9	50 ± 12	35	1
35	15 ± 4	19	18	26 ± 6	27	2
35	49 ± 4	21	21	114 ± 11	48	2

at low temperatures, and the preoviposition period is controlled by egg maturation and not by the presence of spermatozoa in the spermatheca. Seven of the 13 unmated females contained at least one, but never more than six mature eggs per ovariole. The eggs in the remaining six unmated females were less than three-fourths the mature size.

In exceptional circumstances, the preoviposition period could be extended by the absence of spermatozoa in the spermatheca. If a female emerges in an area of low population density, males may not be encountered immediately, and eggs may mature before the female mates and spermatozoa become available.

The availability of suitable food may also be a factor influencing the length of the preoviposition period. Although direct evidence of such effects was not obtained, it can be inferred from the data on the effect of food on the oviposition rates (table 6, p. 437). Females that were offered no food or water matured only 0.02 egg per hour during the first 48 hours of adult life; those with water alone matured 0.3 egg per hour during the first 83 hours of adult life; those with sugar-water matured 1.0 egg per hour during the first 51 hours of adult life. Of the 14 females without food, only two contained mature eggs 48 hours after eclosion. Eleven of the 17 females given water alone, and dissected 83 hours after eclosion, contained mature eggs.

Unfavorable physical conditions can also greatly extend the preoviposition period. Again, direct evidence is lacking, but the effects can be inferred from the effects of temperature on the maturation of eggs (table 1). At 10° C, egg maturation is retarded to such a degree that the adult may die

before the eggs mature. At this temperature, only one of eight females contained any mature eggs 23.8 days after eclosion. The eggs were only one-fourth developed in size in the remaining seven females. Activity at 10° C is very limited, and it is possible that oviposition would not occur even though eggs were mature.

As the temperature is increased, the rate of egg maturation increases. At 15° C, the average preoviposition period is probably around seven days after eclosion; at 25° C, it probably averages around 36 hours. At 35° C, the data indicate the preoviposition period at probably less than a half day.

ENVIRONMENTAL FACTORS INFLUENCING OVIPOSITION RATES

The oviposition rates of many insects are greatly modified by prevailing environmental conditions. If they are favorable, a large number of progeny will be produced in a short period of time, whereas an unfavorable environment will markedly reduce the progeny. Some of the critical environmental factors tending to influence oviposition in insects are temperature, humidity, light, food, and available oviposition sites. These factors may act independently or in combination with one another. For example, in the absence of light, females of *C. philodice eurytheme* will not oviposit, and light at such times influences oviposition independently of temperature. Similarly, very low temperatures may completely stop oviposition. On the other hand, at other levels these factors may interact.

Experimental Procedures

Environmental Control Cabinet. The environmental control cabinet was constructed from a cold-food display case with windows on each side (fig. 4). The interior of the cabinet was 32 inches long, 24 inches wide at the base, and 24 inches high. Twelve inches above the floor, the walls tapered inward to the 8-inch-wide ceiling.

Thirteen fluorescent tubes were mounted within the cabinet (fig. 5). They were arranged in an inverted V over the top and on each side. Each tube was backed by a reflector made from a curved strip of highly polished aluminum. With the 13 lights in operation, approximately 800 foot-candles of light were obtained at any position on the working platform.

A separate unit, including a second refrigerator, was constructed outside the environmental control chamber for humidity control. In this humidifying unit the air was saturated at one temperature, then heated and pumped back into the control chamber to give the desired humidity. Theoretically, by proper selection of saturation temperatures, any humidity could be obtained. In operation at very high and very low humidities, some difficulties were encountered. The cooling system in the control cabinet often operated to remove excess heat generated by the lights. The cooling coils of this system also removed moisture from the air. Thus, it was occasionally necessary to put additional water vapor into the cabinet at high humidities. It was also necessary to exchange the air as rapidly as possible without creating undesirable air currents within the cabinet at low humidities.

For high humidities, the air was pumped out of the control cabinet and into the humidifying unit. There the air was forced through a fine copper screen to insure atomization. The fine air bubbles became saturated as they passed through the heated water bath. The air was then returned to the control cabinet. For low humidities, the water was removed from the humidi-

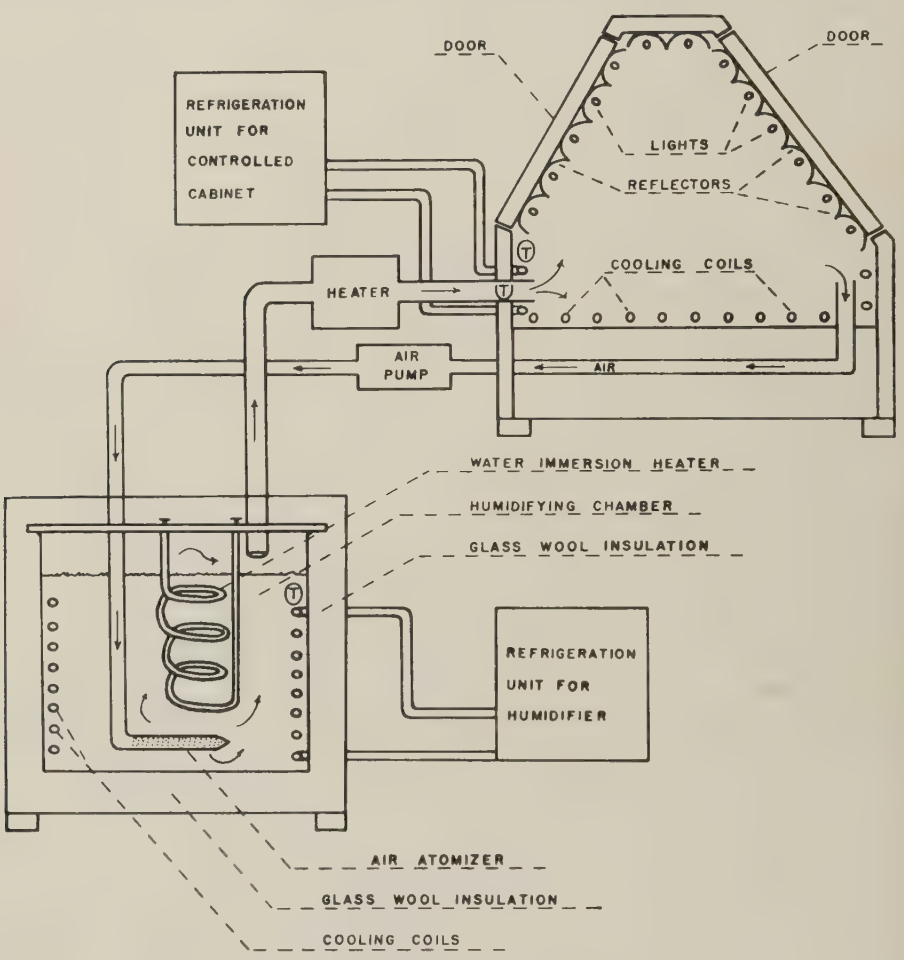

Fig. 4. Schematic drawing of environmental control cabinet.

fying unit, and the refrigerator was put into operation. When cabinet air was passed over the refrigerator coils by means of baffles, its water vapor deposited on the cooling coils. The air containing the desired amount of water vapor was then returned to the control cabinet.

The fluorescent lights produced enough heat to maintain the cabinet at any temperature desired in this study. However, an independent heater was constructed and sometimes used in the system in order to maintain the temperature of the air coming out of the humidifying chamber at a

constant level so that it would hold its water vapor on the way to the control cabinet. One of the principal difficulties encountered was the maintenance of constant humidities while extracting the heat produced by the lights. For further details concerning the construction of this cabinet, see Stern (1952).

Copper-constantan thermocouples were used to determine the body temperatures of female butterflies, black-globe thermometer temperatures, and ambient air temperatures in the environmental control cabinet. The body

Fig. 5. Interior of environmental control cabinet showing one bank of fluorescent lights and the working platform.

temperatures were taken at the conclusion of the tests by inserting a 30-gauge, glass-insulated thermocouple into the thorax. The black-globe thermometers (fig. 6) were 6-inch, copper water-floats painted with Eastman Kodak's No. 4 dull black brushing lacquer. Fifteen minutes were allowed for the black-globe thermometers to come into equilibrium. The ambient air temperature thermocouples were shielded by slightly polished tin cans (fig. 7).

Standardization of Field-collected Females. Attempts to rear large quantities of *Colias philodice eurytheme* females in the laboratory were unsuccessful because of attacks by the polyhedrosis virus and because successful matings were difficult to obtain. Consequently, it was necessary to obtain females from field populations. Essentially, all field-collected females were found to be mated. Whenever possible, the females were collected from fields in which they were emerging. However, a number of groups were collected

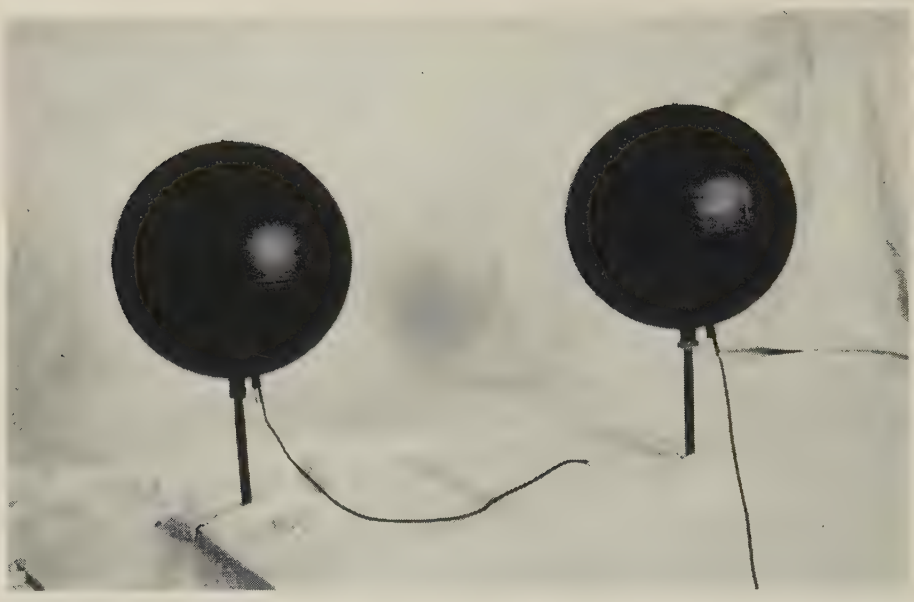

Fig. 6. Black-globe thermometer for measuring effectual radiation temperature.

from oviposition fields when emerging populations were not available. Whatever the source, a random sample of 20 females from each group was subjected to a standard set of conditions. These check butterflies from each field sample were given sugar-water (33 gm cane sugar per 100 cc water) for food, exposed to 800 foot-candles light intensity, a black-globe tempera-

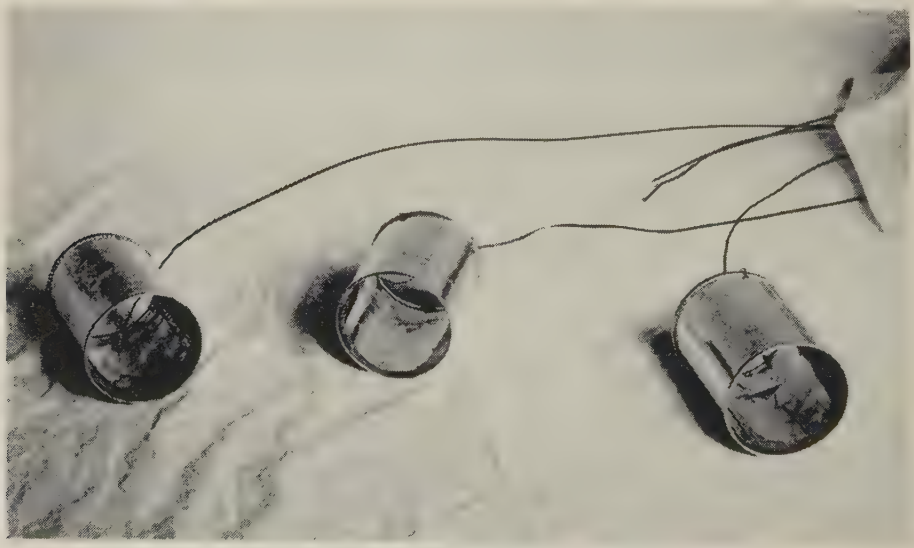

Fig. 7. Polished tin cans used as shields for thermocouples.

ture of 34° C, ambient air temperature of 30° C, and 39 per cent relative humidity. The variation of these checks is shown in table 2.

Since there was considerable variation in the oviposition rates of the groups of females collected on various dates, an oviposition rating was used to facilitate comparison in experiments containing more than one group of females. This rating was based on the performance of the check group for each of the field-collected samples. The check groups were given an oviposition rating of 100. The number of eggs/hour/female given as a percentage of the check is the oviposition rating. The total eggs produced in each test

TABLE 2

OVIPOSITION RATES OF GROUPS OF 20 FEMALE *COLIAS PHILODICE EURYTHEME* EXPOSED TO STANDARD CONDITIONS*

Date collected	Field stage	Total eggs laid	Total test hours	Eggs per female per hour
6/10	oviposition	1,432	130	11
6/18	oviposition	2,115	183	12
6/25	oviposition	2,925	144	20
7/10	oviposition	5,699	177	32
9/12	oviposition	2,485	132	19
7/2	emergence	4,161	147	28
7/17	emergence	4,765	88	54
8/7	emergence	5,473	150	37
8/21	emergence	5,007	179	28
9/7	emergence	3,802	113	34
9/19	emergence	2,538	72	35
9/25	emergence	2,555	105	25
10/1	emergence	1,785	139	13

* Ambient air temperature, 30° C; black-globe thermometer temperature, 34° C; 39 per cent relative humidity; light, 800 foot-candles; food, sugar-water.

were divided by the number of adult hours. The adult hours were considered to be the number of hours of exposure. Thus, 20 adults in the cabinet for two hours equaled 40 adult hours. To obtain the oviposition rating for any particular test, the number of eggs/hour/female produced by the check females was divided into 100 times the eggs hour female of the test females;

$$\text{oviposition rating} = \frac{100 \times \text{test rate}}{\text{check rate}}$$

Testing Conditions. For each series of tests, the females were brought into the laboratory in an ice box and released into a large cage. From this large cage, either five white or five yellow females were selected at random and each group of five was placed in a single wire cage. Four such cages, making a total of 10 white and 10 yellow females, were used for each test. These cages (figs. 8 and 9), made from galvanized wire cloth (size 3 mesh, No. 21 wire), were 3¾ inches in diameter and 3½ inches tall.

Alfalfa was used as an oviposition host for all tests on reproductive performance. Each day a fresh bouquet of two or three growing tips of alfalfa was placed in each cage just before starting the test, and taken out immediately after the test. The eggs on each bouquet were then counted and recorded.

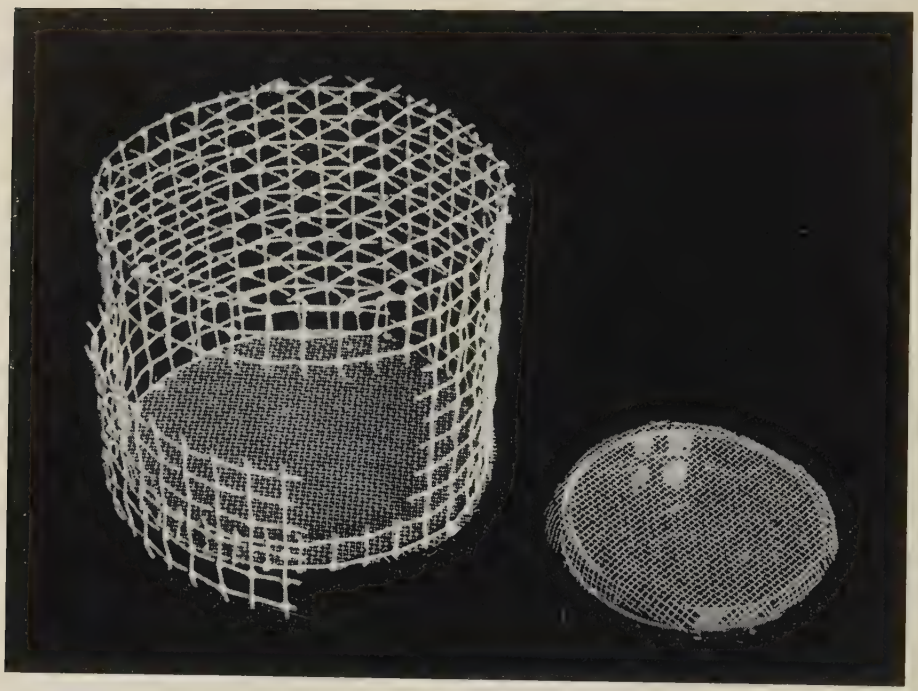

Fig. 8. A single cage with a food dish.

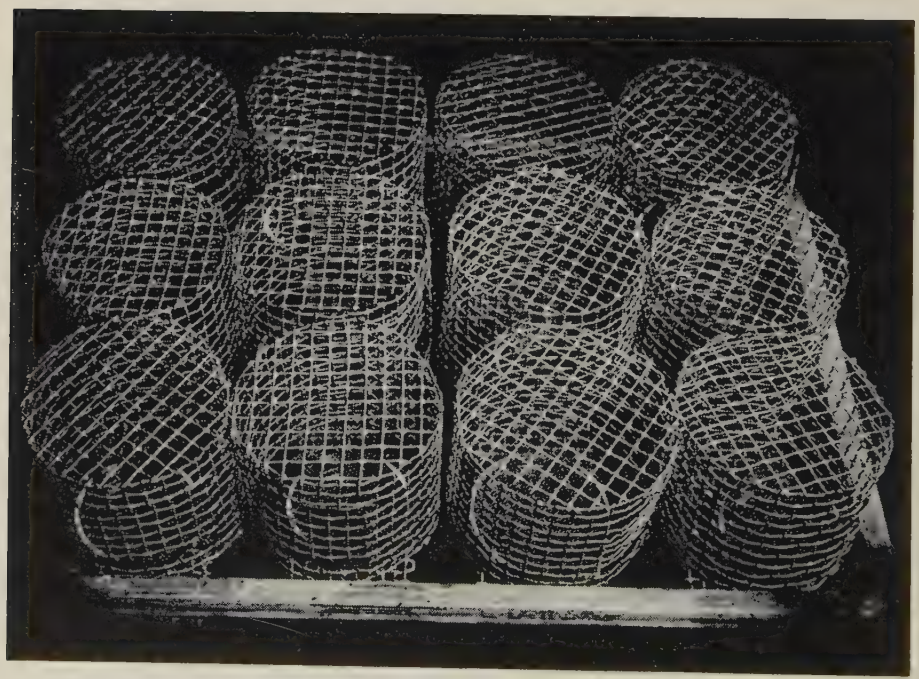

Fig. 9. The working platform with 12 cages.

In nearly all experiments, the females were exposed to the test conditions for three-hour periods on three successive days. The females were held in the dark at 25° C and 80 per cent relative humidity while not under test conditions. Females do not oviposit in the dark unless a large number of eggs have matured. Even then, oviposition appears to be a matter of pressure rather than a voluntary function. At the end of a test, the females were discarded, and a new group was used for the next test. The hours of exposure, eggs laid, and number of surviving females were recorded each day. Any female that died during the day of the test run was not considered to be present in the totaling of adult hours for that day.

A number of experiments were conducted with variations in the numbers of females placed in the individual cages, length of exposure to test conditions, and holdover temperatures. These tests were conducted with 20 females (10 yellow and 10 white). There was essentially no difference in oviposition (eggs/hour/female) with either three or five females in a single test cage. When five females were placed in a cage, they oviposited 32 eggs/hour/female, while with three females per cage, 30 eggs/hour/female were oviposited. In the test for total oviposition, individual females were placed in a cage. Under these conditions, oviposition dropped to 19 eggs/hour/female during the test period. (All other tests were started with five females per cage.) Apparently, numbers of adults in a single cage increase the activity, resulting in more eggs oviposited. This factor may have had some effect on the oviposition rating at extremely high temperatures, when mortality rapidly reduced the numbers of females during testing conditions. However, the effect is considered to be small.

To determine differences, if any, between lengths of exposure to test conditions, various groups of females were exposed to test conditions for one, three, six, and 10 hours. There was essentially no difference between oviposition rates of females exposed for one hour and those exposed for three hours. When exposed for one hour, 26 eggs/hour/female were oviposited, while an exposure of three hours gave 25 eggs. At the longer exposure periods of six and 10 hours, oviposition dropped to 21 and nine eggs/hour/female, respectively. In the laboratory experiments, the females were generally exposed to testing conditions for three hours on three successive days. In no case were the females exposed to the test for longer than three hours. In some instances less time was allowed, but never less than one hour. In addition, females were exposed to one, two, and three hours of testing conditions every day and every other day for three and five days, respectively. At exposures of one, two, and three hours every day, 22, 26, and 24 eggs/hour/female were oviposited, respectively, while at one, two, and three hours of exposure every other day, 46, 32, and 29 eggs/hour/female were oviposited, respectively. In the laboratory experiments, the tests were nearly always conducted on three successive days. On rare occasions when a day was missed, the test females were placed at 15° C for that day. At that temperature, oögenesis essentially stops.

Tests were also conducted on temperature conditions during the holdover period, that is, the time at which the females were not exposed to testing conditions. At 24° and 35° C, the eggs/hour/female were 17 and 18, respec-

tively. The holdover conditions for all laboratory tests were 24° C at 80 per cent relative humidity, with no light when the tests were conducted on successive days.

Ambient Air and Body Temperatures

The daily cycle of body temperatures of most poikilotherms generally follows a trend much the same as that of air temperature, but the body temperature of an insect rarely coincides with the ambient air temperature. The body temperature, like the air temperature, oscillates a few degrees during the daily cycle. During the night, however, the fluctuations are dampened. The body temperature of many terrestrial insects living in an exposed environment may differ considerably from the ambient air temperature (Kennedy, 1939; Wellington, 1949; Pepper and Hastings, 1952; Leigh and Smith, 1959; and many others). The variations in the difference between body temperature and ambient air temperature may be so large that continuous records of air temperature alone may be of little value (Wellington, 1949). This is worthy of consideration not only in the field, but in the laboratory as well. The wall temperatures of cabinets may be independent of the air temperature and may radiate energy which may have a marked effect on the body temperature of insects in the cabinets. Moreover, caution must be used when applying data gained from laboratory temperature cabinets to field conditions on the assumption that the air and body temperatures from cabinet data are comparable with air and body temperatures in the field.

Both the plant habitats of insects and the insects themselves behave more or less as black bodies. The black-globe thermometer, used by various researchers (Vernon, 1932; Bedford and Warner, 1934; Bond and Kelly, 1955), offers a better indication of the insect body temperature than does air temperature (Leigh and Smith, 1959). The temperature of the black-globe thermometer depends upon the environment in which the instrument is placed. If the surface of objects surrounding the black-globe thermometer is warmer than the air, the temperature recorded will be above air temperature; conversely, with surroundings cooler than the air, the instrument will record below air temperature. When the black-globe thermometer is in equilibrium with its environment, the effects of radiation, conduction, and convection balance each other. The black-globe temperature is an indication of the combined effects of radiant energy, ambient air temperature, and air velocity (Bond and Kelly, 1955). Poikilothermous animals such as insects will have heat exchanges comparable with those of the black-globe. The body temperature of an insect will differ from that of the black-globe thermometer because of activities of the insect and differences in physical characteristics, particularly heat capacity and shape. Nevertheless, the black-globe temperatures and the mean radiant temperatures and radiant heat loads derived from the black-globe data are useful and significant in environmental research.

In the control cabinet, the black-globe thermometer and body temperature were nearly the same at all air temperature settings below 34° C. When the black-globe temperatures were 34° and 38° C, the body temperatures were 32° and 36° C, respectively (table 3). There was generally a difference of two to four degrees between the body and air temperature, except at the

higher air temperature (46° C) at which point body and air temperatures were the same (table 3).

Temperature and Oviposition Rates

Although temperature is only one factor in the environment of an insect, it is omnipresent and is especially critical for poikilothermic animals. If, at any time, the body temperature is not within favorable limits, the or-

TABLE 3

OVIPOSITION RATES OF *COLIAS PHILODICE EURYTHEME* AS AFFECTED BY TEMPERATURE AND HUMIDITY WHEN SUGAR-WATER WAS GIVEN AS FOOD

Relative humidity	Temperature			Total eggs laid	Total test hours	Eggs per hour per female	
	Black-globe	Body	Air			Actual	Oviposition rating
per cent	°C	°C	°C				
8–16	34	32	30	947	123	7.7	27
	38	36	34	1,207	113	10.7	44
	42	42	39	418	98	4.3	15
	45	46	46	34	67	0.5	2
21–24	28	28	25	1,436	96	14.9	42
	34	32	30	2,244	112	20.0	56
	38	36	34	3,183	126	25.4	79
	42	42	39	2,004	122	16.4	46
26–30	21	21	17	37	95	00.4	1
	26	25	22	2,049	105	19.5	60
	34	32	30	5,601	112	50.6	94
	38	36	34	2,473	153	16.1	66
36–45	14	14	10	37	130	0.3	1
	21	21	17	304	164	1.9	9
	26	25	22	2,145	177	12.1	110
	28	28	25	1,928	117	17.0	90
	34	32	30	2,115	183	11.6	100
	38	36	34	1,555	104	15.0	79
	42	42	39	944	164	5.8	50
	45	46	46	3	64	0.1	1
50–63	26	25	22	1,777	152	11.7	35
	34	32	30	1,916	120	16.0	48
	38	36	34	2,008	112	17.9	95
	42	42	39	1,038	123	8.4	25

ganism may fail to survive, grow, or reproduce although all other conditions are favorable.

Temperature was found to be more important than humidity, food, or light intensity in causing variation in the oviposition rates of *C. philodice eurytheme*. Body temperatures for high oviposition rates extended over a range of approximately 28° to 36° C in environments with relative humidities ranging from 26 to 45 per cent (table 4 and fig. 10). Within this temperature range (28° to 36° C), 32° C appeared to be the most desirable temperature for high oviposition. Although the data in table 4 do not clearly

TABLE 4

SUMMARY OF RELATIVE OVIPOSITION RATINGS OF *COLIAS PHILODICE EURYTHEME* FEMALES AT VARIOUS BODY TEMPERATURES AND HUMIDITIES, TESTED UNDER UNIFORM CONDITIONS*

Range of relative humidity	Oviposition ratings at following body temperatures (° C):							
	14	21	25	28	32	36	42	46
per cent								
8–16	..	..	..	..	27	44	15	2
21–24	..	..	42	..	56	79	46	..
26–30	..	1	61	..	94	66	..	..
36–45	1	9	110	90	100	79	50	0.5
50–63	..	..	35	..	48	95	25	..

* Light intensity, 800 foot-candles; food, sugar-water.

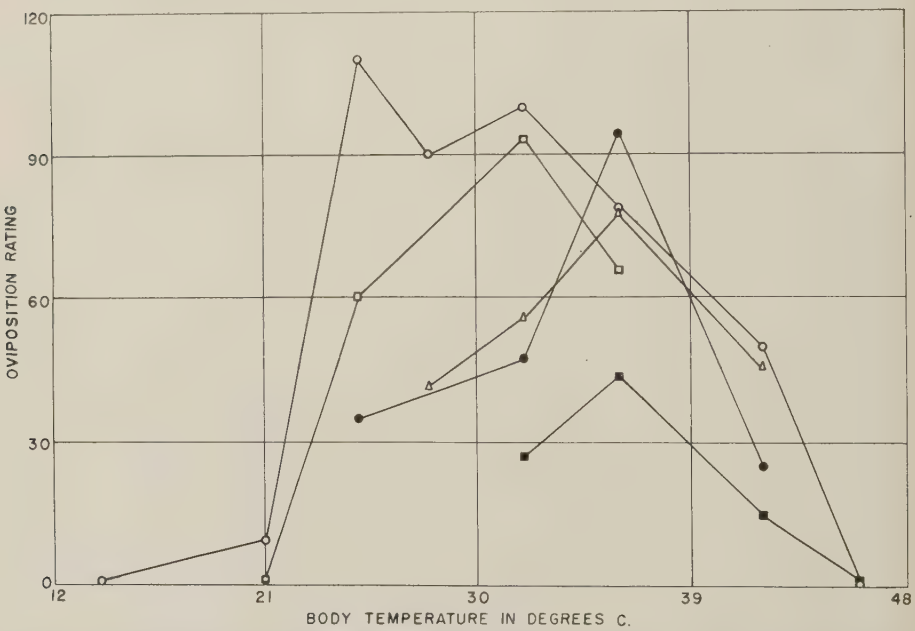

Fig. 10. Influence of body temperature on oviposition ratings of *Colias philodice eurytheme* at relative humidities of 8 to 16 per cent (solid squares), 21 to 24 per cent (open triangles), 26 to 30 per cent (open squares), 36 to 45 per cent (open circles), and 50 to 63 per cent (closed circles).

indicate this, other data support the statement. The supporting data are based on the check samples of females exposed to conditions producing a body temperature of 32° C (humidity 39 per cent). With but one exception, irrespective of the humidity, food, or light intensity, the oviposition rating of the test butterflies was always lower than the check sample exposed to 32° C.

At humidities below 24 per cent, the females with body temperatures of 36° C showed a higher oviposition rating than did females at 32° C (table 4). Within a humidity range of 50 to 63 per cent, the oviposition rating at

36° C body temperature was nearly twice as large as at 32° C. Viewing the oviposition ratings at other body temperatures (table 4), within the same humidity range, the oviposition rating for 36° C suggests some error. It should probably have been between 40 and 50.

Body temperatures near 46° C and 21° C appear to be the upper and lower limits of oviposition. At 46° C the females die rapidly. Although mortality is not increased, at a body temperature of 21° C activity as well as oviposition is markedly decreased. The adults hang on the sides or rest on the bottom of the cage. This lower thermal limit for oviposition (body temperature 21° C) corresponds to the ambient air temperature of 17° C. The oviposition rating recorded at a body temperature of 25° C and 36 to 45 per cent humidity range appears to be too high.

Humidity and Oviposition Rates

For maximum oviposition rates relative humidity appears to range from 26 to 45 per cent. As the humidity is increased or decreased beyond this range, the oviposition rate generally decreases (table 4, fig. 10). With a constant body temperature of 32° C, the oviposition rating within the humidity range of 36 to 45 per cent was nearly four times as high as at a range of 8 to 16 per cent. Within the 50 to 63 per cent range, the oviposition rating was half as high as within the 36 to 45 per cent range. With a humidity range of 21 to 24 per cent, the oviposition rating was comparable with the 50 to 63 per cent range.

At extremely high (46° C) and low body temperatures (21° C), any effect that humidity may have on oviposition is masked by the effects of temperature. At body temperatures approaching the upper thermal limit (46° C), it is likely that humidity has little effect on oviposition although, at this high temperature, a very low humidity may tend to increase the mortality rate of females through desiccation. At the lower temperatures, activity is markedly reduced, and any effect that humidity may have on the oviposition rate is again masked by the low temperature.

Light and Oviposition Rates

Females do not oviposit in the absence of light unless they are held constantly in the dark for a number of days. When eggs are laid in the dark, oviposition appears to be involuntary as the result of mechanical pressure, rather than voluntary. Light intensities of 800, 600, 400, 200, and 100 foot-candles were compared (table 5). As the light intensity decreased, the black-globe temperatures and body temperature also decreased, while the air temperature remained constant. One exception was that, at 800 foot-candles and a globe thermometer temperature of 34° C, the air temperature was 30° C. At the other light intensity settings, the air temperature remained near 27° C. Unfortunately, it was not possible to maintain the humidity at the same level with the equipment available. A decrease in light intensity within the 100 to 800 foot-candle range appeared to have no consistent effect on oviposition ratings when light was considered without respect to temperature and humidity.

Oviposition Under Field Conditions

On hot summer days, oviposition occurs principally during the morning, but it also takes place in the late afternoon. During midday, very few eggs are laid. The females spend most of the time seeking food or resting on alfalfa stems parallel to the sun's rays (Leigh and Smith, 1959). Field observations further indicated that there is a general decrease in oviposition during the afternoon hours in comparison with the number of eggs oviposited during the morning. Since the female will not oviposit in the dark, the eggs she lays during the morning hours are those that matured during the night. Eggs laid in the afternoon are mostly those maturing after the heavier morning oviposition. These findings were also demonstrated in the laboratory tests.

TABLE 5

INFLUENCE OF LIGHT INTENSITY ON OVIPOSITION RATINGS OF *COLIAS PHILODICE EURYTHEME*

Light intensity	Temperature			Relative humidity	Total eggs laid	Total test hours	Eggs per hour per female	
	Black-globe	Body	Air				Actual	Oviposition rating
foot-candles	*° C*	*° C*	*° C*	*per cent*				
800	34	32	30	53	1,916	120	16	48
600	30	30	27	54	1,747	194	9	70
400	28	29	27	62	3,258	106	31	57
200	27	27	27	74	3,261	155	21	58
100	25	26	26	76	1,549	169	9	71

Environmental conditions in oviposition fields tend to coincide with the optimum air temperature and humidity that gave the highest oviposition rating in the control cabinet (Leigh and Smith, 1959). Under Central Valley conditions, the oviposition fields with a higher air temperature and lower humidity than that of emergence fields are usually more favorable for oviposition than the cooler, more humid, emergence fields.

As indicated by Leigh and Smith (1959), light intensity may be limiting to flight and probably to oviposition under field conditions. During their investigations, they found temperature, light, and humidity conditions most favorable for flight and oviposition during the morning and afternoon in the hot summer period, and near midday in the spring and fall.

Nutrition and Oviposition Rates

An adequate food supply during some stage in the life cycle of an organism is essential for survival, growth, and reproduction. If the larvae of this species are not continually supplied with abundant food, they produce small adults which often die soon after emergence. If alfalfa fields are cut early, many of the larvae in the fifth instar pupate prematurely, but the resulting adults are often malformed, or fail to emerge successfully.

Carbohydrates, and probably other nutrients, are essential in the female diet for high oögenesis (table 6). In the field, food is supplied from the nectaries of flowers. Often adults can also be seen feeding from wet spots along irrigation ditches or from ponds. Samples show that these adults are nearly all males. This type of feeding may be to replenish water loss resulting from evaporation and respiration in the male, but it does not serve as a regular food supply for the female.

Just before eclosion, the fat body becomes a distinguishable mass of tissue. As eggs mature, the size of the fat body decreases. In very old females,

TABLE 6

MATURATION OF EGGS AND CHANGES IN SIZE OF THE FAT BODY IN NEWLY EMERGED *COLIAS PHILODICE EURYTHEME* FEMALES UNDER VARIOUS CONDITIONS*

Diet	Hours from eclosion to dissection†	Number dissected	Mean fat body rating‡	Females with mature eggs	Mean number of mature eggs per female
No food or water	48	14	4.0	2	0.9
	72	24	3.1	15	18
	144	20	2.8	11	15
Water only	83	17	3.7	11	28
	163	20	2.6	16	53
Sugar-water	6–24	24	4.0	1	0.4
	51	10	3.8	9	50
	83	15	3.3	15	97
	109	10	3.4	10	106
	164	13	2.3	13	157
	229	11	2.0	11	188

* Temperature, 25° C; relative humidity, 80 per cent; no light.
† Samples were usually dissected in a period of three or four hours, depending on number in sample.
‡ 4 = highest rating.

scarcely any of the original tissue is visible. If adults are not given sugar in their diet, the fat body decreases rapidly, and few eggs are produced (table 6). Apparently, if the female is not supplied with sugar in the diet, the energy stored in the fat body can be used for general activities in addition to egg production.

Effect of Food on Oögenesis. The supply of adult food has a definite effect on oögenesis, fat body size, and reproductive performance of *Colias philodice eurytheme*. One experiment was conducted to test the effect of no food, water only, and sugar-water (33 gm cane sugar to 100 cc water) on oögenesis (table 6). Pupae were collected from an alfalfa field, and brought into the laboratory. As the females emerged, they were placed in small cages in a cabinet, at 25° C, with a relative humidity of 80 per cent. After a given number of hours, groups of the females were dissected, and the number of mature eggs was recorded.

There are no mature eggs in the females at the time of emergence; thus, the number of eggs recorded at the time of dissection matured after eclosion. Individuals do not emerge simultaneously, and a variation of ± 4 hours

was therefore allowed in calculating the total hours from eclosion. On dissection, the fat body content was visually rated from 4 to 0. A number 4 rating was highest, and is characteristic of females which have recently emerged. A totally expended fat body was given a zero rating.

Groups of females that were allowed no food were dissected 48, 72, and 144 hours after eclosion. The test was not carried beyond 144 hours because some of the females in this group were nearly dead after that length of time. In this test (no food), not only were a low number of eggs produced per female after 144 hours, but the fat body was also greatly decreased in size. The fat body after 144 hours is comparable with that of females held for 163 hours with water as food and 164 hours with sugar-water as food. However, in comparing eggs produced, females given no food contained an average of 15 eggs per female after 144 hours; females given water averaged 53 eggs per female after 163 hours; and females given sugar-water contained 157 eggs per female, 164 hours from eclosion.

The females offered no food contained 18 eggs per female 72 hours after eclosion, while the group dissected after 144 hours contained 15 eggs per female. The standard error of the mean of the two groups was 5.0 eggs per female for those dissected after 72 hours, and 4.5 eggs per female for those dissected after 144 hours. This overlap of egg production may indicate that females offered no food had essentially reached their maximum egg production approximately 72 hours after eclosion. Moreover, mortality becomes a factor after 144 hours without food, and all of these females would have died prematurely without approaching their potential fecundity.

The females given water only produced more eggs per female than did those given neither food nor water in a comparable length of time, although the fat body ratings were similar at the time of dissection.

The females given sugar-water matured more eggs in less time than did females given either water or no food. There was a gradual decrease in fat body size in the sugar-water fed females as egg production increased, while those females offered no food had a decrease in fat body size with very little egg production. Those females given water only had a decrease in fat body as the production of eggs increased, but the egg production was not nearly so high as that of the sugar-water group.

From the results obtained, it can be stated that, for high fecundity and longevity, moisture alone is not sufficient, and some carbohydrate is required.

Effect of Food on Oviposition. A carbohydrate diet is essential for high oviposition as well as increased longevity.

The females used for the study of nutrition as it affects oviposition were collected from the field, and had undoubtedly taken some nectar containing sugars and other nutrients. In spite of this, however, some information was gained, inasmuch as the nutritional studies on oviposition supported the data gained from the studies on oögenesis.

Nutrition tests were conducted with an 800 foot-candle light intensity and a humidity range of 36 to 45 per cent. The body temperatures in this series of tests were 14°, 21°, 26°, 28°, 32°, 36°, 42°, and 46° C. The females were allowed either sugar-water, water only, or no food (table 7, fig. 11).

Females given sugar-water oviposited more eggs than did females given

no food. Differences between females given sugar-water and those given water only were negligible at the lower and higher body temperatures. At body temperatures of 25°, 28°, and 32° C, the females given sugar-water oviposited more eggs than did those given water only. As mentioned previously, all the females had undoubtedly taken food in the field, a fact which may have masked the full effect of the different diets used in this test.

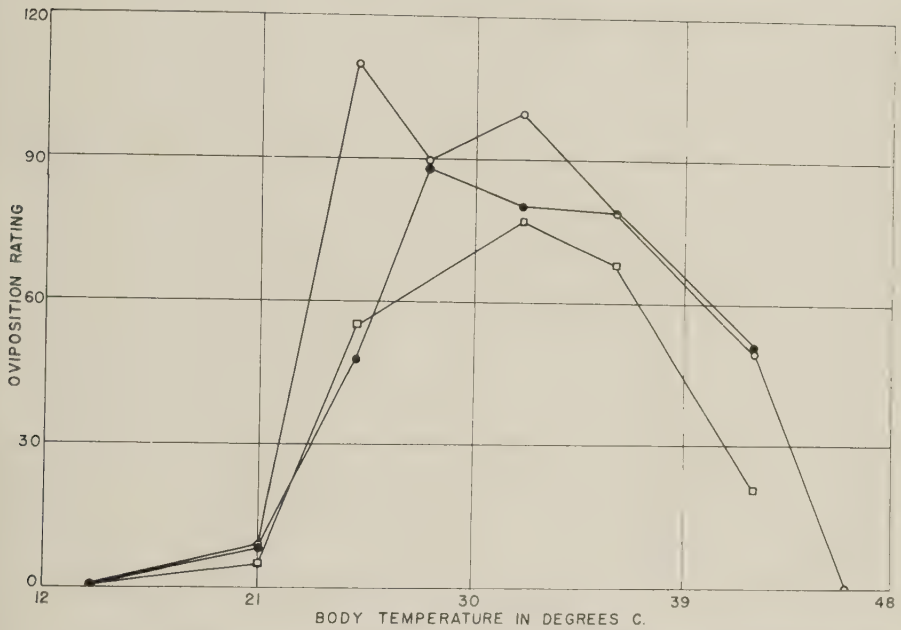

Fig. 11. Influence of body temperature on oviposition ratings of *Colias philodice eurytheme* when offered sugar-water (open circles), water only (solid circles), and neither food nor water (open squares).

Available Oviposition Sites

An abundant supply of oviposition sites is usually available to *Colias philodice eurytheme* where alfalfa is grown commercially. Occasionally the supply of suitable oviposition-stage fields may be low, and oviposition rates may be affected. The quality of alfalfa fields as oviposition sites is reduced as the fields mature and, as a result, there is considerable differential oviposition among fields (Smith, Bryan, and Allen, 1949).

Before the introduction of alfalfa and its extensive use as a forage crop, the host plants were more widely scattered in ravines, sloughs, or along river banks. As a result, oviposition was undoubtedly affected, and full reproductive potentials were seldom realized. When oviposition sites are scarce, eggs may be fertilized long before such a site is found. In the sparsely vegetated areas of California, as well as in northern or elevated areas, the paucity of oviposition sites undoubtedly is a factor in reducing oviposition.

TABLE 7

OVIPOSITION RATES OF *COLIAS PHILODICE EURYTHEME* AT VARIOUS TEMPERATURES AND HUMIDITIES,* AS AFFECTED BY AVAILABLE FOOD

Temperature			Sugar-water				Water only				No food or water			
Black-globe	Body	Air	Total eggs laid	Total test hours	Eggs per hour per female		Total eggs laid	Total test hours	Eggs per hour per female		Total eggs laid	Total test hours	Eggs per hour per female	
					Actual	Oviposition rating			Actual	Oviposition rating			Actual	Oviposition rating
°C	°C	°C												
14	14	10	37	130	0.3	1	21	126.5	0.2	0.4	11	126.8	0.1	0.3
21	21	17	304	164	2	9	267	152.0	2	9	153	146.5	1	5
26	25	22	2,145	177	12	110	1,526	155.0	10	48	1,350	120.0	11	56
28	28	25	1,988	117	17	90	1,923	115.4	17	88	.....	.....	..	..
34	32	30	2,115	183	12	100	851	91.5	9	81	1,645	104.5	16	77
38	36	34	1,555	104	15	79	1,524	101.5	15	80	1,209	94.5	13	68
42	42	39	944	164	6	50	698	118.5	6	51	341	136.0	3	22
45	46	46	3	64	0.05	0.5	.....	.....	..	..	.....	.....	..	..

* Relative humidities ranged from 36 to 45 per cent.

POPULATION PHENOMENA INFLUENCING OVIPOSITION RATES

The oviposition rates of a population of adult *Colias* will be determined by its size, the environmental conditions prevailing, the fecundity of the females, the sex ratio, and the age distribution of the population.

The size of the population existing in a particular alfalfa field is the result of a complex of ecological and historical factors that will not be discussed here (see Smith and Allen, 1954; Smith, Bryan, and Allen, 1949; Allen and Smith, 1958). The influence of the environmental conditions on oviposition has already been discussed.

Fecundity

Wildermuth (1921), studying the biology of this species in Arizona, reported that a female may lay from 200 to 500 eggs. In order to gain further information of individual and population oviposition, a sample of over 100 females was collected from an emergence field in late October. Thirty-five of these were selected at random and placed in individual cages. Each day a fresh bouquet of alfalfa was placed in each cage, and the eggs laid were recorded. At the environmental conditions used in this test (black-globe temperature 34° C, air temperature 30° C, light intensity, 800 foot-candles, and a relative humidity of approximately 40 per cent) the body temperature was 32° C. All of the females were offered sugar-water as food, which consisted of 33 gm of cane sugar in 100 cc of water. Each cage was placed in the control cabinet for three hours a day. On two occasions it was not possible to expose the butterflies to the test conditions for a full three hours. During the time that the females were not exposed to test conditions, they were placed at 25° C, 80 per cent relative humidity, with no light. Occasionally it was impossible to subject the females to test conditions every day. The females were then taken out of the holdover cabinet, i.e., 25° C, 80 per cent relative humidity, and placed at 16° C, 80 per cent relative humidity, with no light. At this low temperature, egg maturation is greatly retarded. Thus, a more precise picture of daily egg maturation and oviposition was obtained than if the females were allowed to maturate a larger number of eggs at 25° C on the days they were not allowed to oviposit. However, there was a slight increase in oviposition after two days of nonexposure to possible oviposition.

To determine the approximate age of the females used in this test, 25 were dissected as soon as they were brought into the laboratory. Of the 25, only nine contained mature eggs at the time of dissection. These 25 females had a mean fat body rating of 3.6. This rating, plus the absence of mature eggs in 16 of the 25 females, indicates that they had recently emerged and that the majority had laid few, if any, eggs prior to their capture.

Of the 35 females used in the test (table 8) on reproductive potential, 17 were phenotypically yellow, 18 were phenotypically white. The range in fecundity under these conditions was from a minimum of 140 eggs, laid by a yellow female which died six days after the start of the test, to a maximum of 1,172 eggs laid by a white female which died after 24 days. One yellow female lived a total of 37 days and laid 829 eggs during that period.

Those females that lived longest did not necessarily lay the largest numbers of eggs.

As each female died, it was dissected, and its fat body content was visually rated from 4 to 0. The combined 35 females had a fat body rating of 0.6 at the time of death, indicating that nearly all the fat body is exhausted after total oviposition.

On the first day of the test, 18 of the 35 females (fig. 12) oviposited, laying a total of 663 eggs (average, 36.6). On the second day, 30 adults laid a total of 2,319 eggs (average, 77.3). The increase in oviposition on the second day of exposure further indicated that most of the females in the sample had recently emerged and had laid few, if any, eggs.

In general, oviposition remained at a high level from the second through the ninth day of the test. After the ninth day, oviposition gradually de-

TABLE 8

FECUNDITY OF *COLIAS PHILODICE EURYTHEME* FEMALES THAT HAD A BODY TEMPERATURE OF 32° C UNDER CERTAIN LABORATORY CONDITIONS*

Phenotype	Number of females	Longevity		Number of eggs per female	
		Mean	Range	Mean ± S.E.M.	Range
		days	*days*		
Yellow	17	22.2	6–37	760.5 ± 18.8	140–1,125
White	18	21.3	7–34	672.6 ± 22.7	198–1,172
Total	35	21.8	6–37	715.3 ± 45.8	140–1,172

* Testing exposure to black-globe temperature of 34° C; air temperature, 30° C; light intensity, 800 foot-candles; relative humidity, 40 per cent; food, sugar-water.

creased until the last female died. The peak of oviposition does not occur immediately after emergence, but requires a few days to reach the maximum.

We do not believe that restriction of the oviposition period to three hours per day greatly modified the daily egg production. General observations plus laboratory data (see "Testing Conditions") indicate that, under the conditions of the test, three hours was sufficient time for the females to lay their daily crop of eggs.

Under optimum field conditions, the high rates of oviposition by populations might be extended over a longer period than nine days, since females would be emerging each day.

However, rarely will all environmental conditions be optimum for high oviposition. During the cooler months of the year, the number of eggs laid per day per population unit is not so high as during the warmer months, because emergence is extended over a longer period, and the rate of oögenesis is retarded. The oviposition of a population unit during autumn months may also be decreased because adequate food, a critical factor in oviposition, may not be available. Further, if the weather turns cold, egg production is decreased even though the longevity of the population unit is extended.

During the hot summer months, a population tends to lay a greater number of eggs per day than during cool months. This is frequently observed in

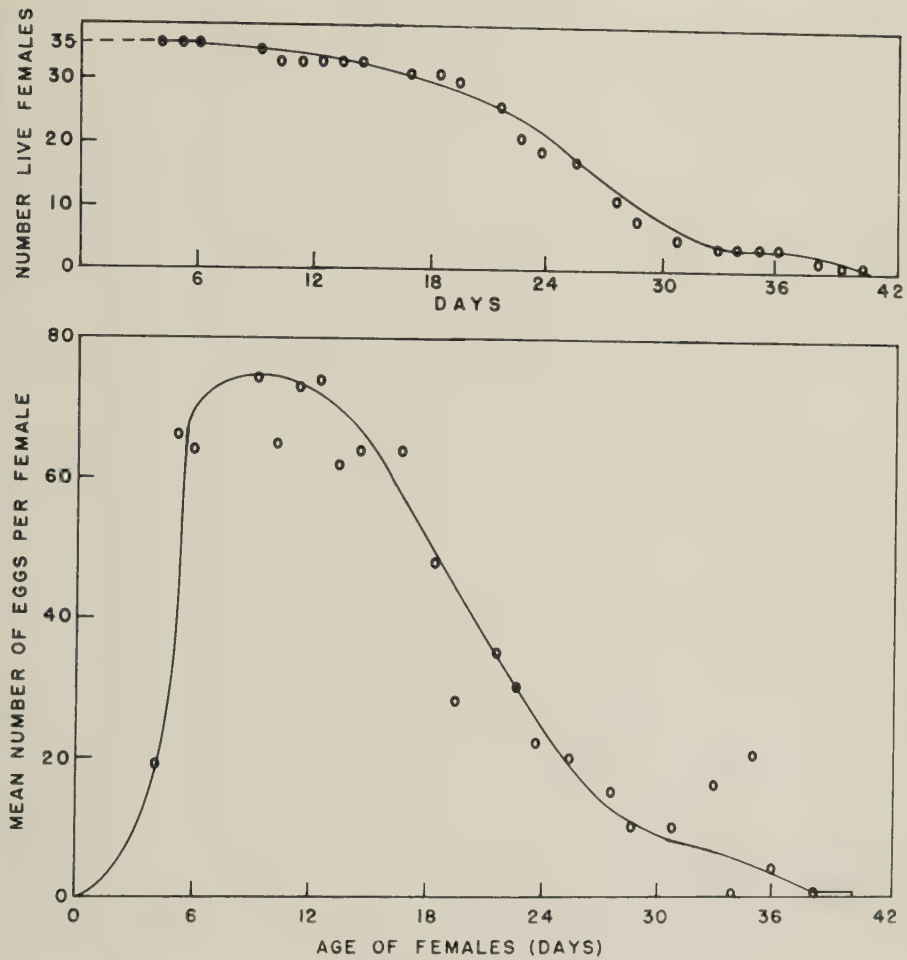

Fig. 12. Relationship of female age to rate of oviposition.

the San Joaquin Valley, California. Larval populations containing only two or three instar stages will often be found. In such fields, there is often but one source of females for oviposition. On the other hand, females from different emergence fields may concentrate in a single oviposition-stage field, but at slightly different times. In this type of field, larvae of all instar stages may be found.

Sex Ratio

In a normal population, the difference in time between the peaks of emergence of the two sexes will be dependent upon the temperature prevailing during larval and pupal development. Theoretical diagrams of emergence at different temperatures are shown in figure 13. At developmental temperatures above 25° C, the difference in time between the peaks of the male and female emergence will probably be less than one day; at lower develop-

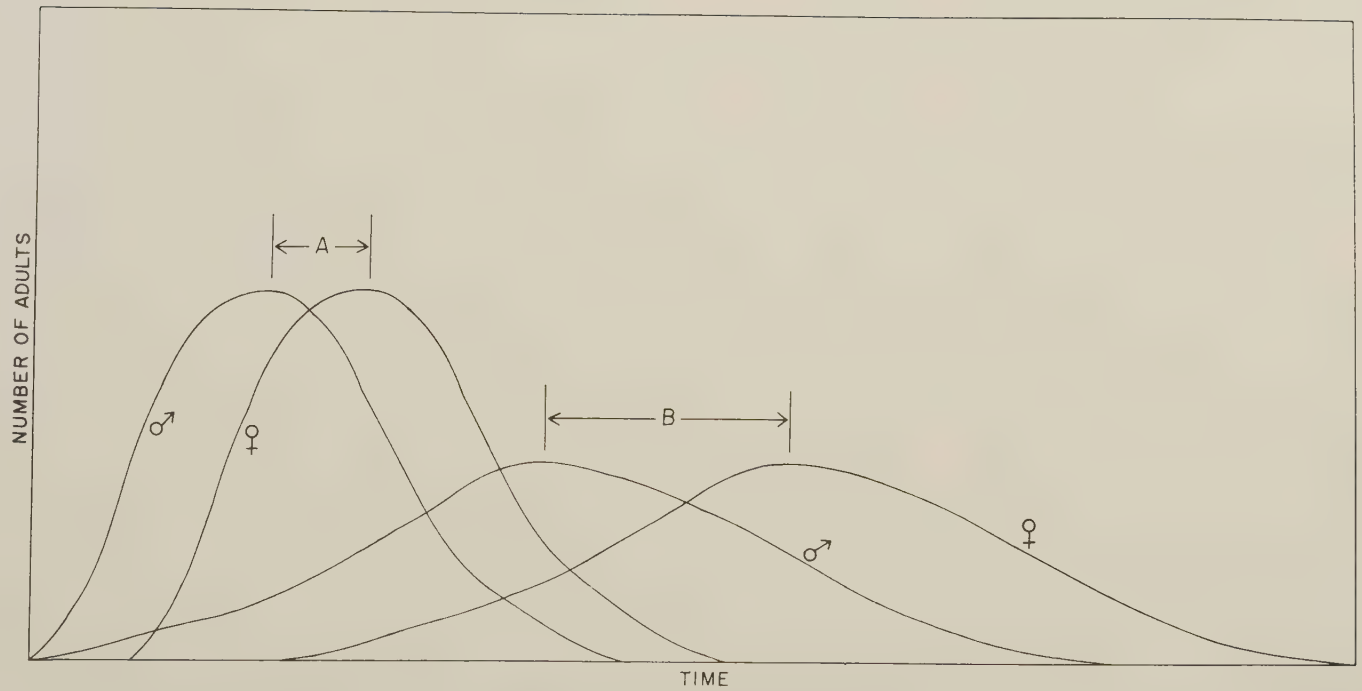

Fig. 13. Theoretical emergence curves at two different temperatures. A. Differential emergence peaks are less than one day apart when developmental temperatures are above 25° C. B. Differential emergence peaks are more than two days apart when developmental temperatures are above 15° C.

mental temperatures, the peaks will be more than one day; and at very low developmental temperatures, the peaks may be a number of days apart.

Sex ratios of this species, obtained by various workers, indicate that generally the males are more abundant than females. Gerould also found this to be true with *Colias philodice philodice* Godart (1923), and obtained the following sex ratios in *C. philodice eurytheme:* 4:1, 2.8:1, and 3.8:1, with the male being more abundant in each case (1946). In rearing *C. philodice philodice* broods in the laboratory, Gerould (1911*b*) found that 82 per cent of the first half of all broods reaching the pupal stage were males. When larvae were subjected to starvation or disease, the males were favored over the females.

Field observations indicate that the female with a slightly slower developmental period may be more subject to attack by the polyhedrosis virus than is the male. For example, in an alfalfa field near Dos Palos, California, in late September, so many males were present that their fluttering wings could be heard. Essentially, no females could be seen in the field. Females tend to leave emergence fields soon after mating, but there are always some remaining or some that have recently emerged. In this field, it is believed that polyhedrosis virus had removed the more slowly developing females from the emerging population as well as some of the males. A sample of 37 normal appearing pupae was selected. Twelve males and two white females eclosed on the first day of emergence, two females eclosed the second day, while the remaining 21 pupae developed wilt and failed to eclose (table 9).

In other situations, unknown factors modified the sex ratio. A random sample of 362 pupae was collected near Westley, California, in late August. There was no evidence of the polyhedrosis virus attacking the population, and only a few adults were present. Cast pupal cases were difficult to find in comparison with the pupae present, and larvae were also very scarce. From a sample of 362 pupae, 206 were males, of which three failed to emerge, while 156 were females, of which four failed to emerge. Some unknown factor may have reduced the more slowly developing female population before pupation. The daily emergence and numbers of each sex from this sample are shown in figure 14.

Another random sample of 467 pupae was taken near Westley, California, during the middle of September. There had been essentially no emergence from the field prior to sampling, but a few fifth instar larvae remained and some of these were attacked by the polyhedrosis virus. In this sample, 271 males emerged, while only 186 females emerged, giving a sex ratio of 1.5 males to one female (table 9). Ten of the pupae failed to emerge. Since a few larvae were in the field, the sample may have favored a selection of the males which pupate earlier, but this would not account for the great difference in sex ratio. Again, polyhedrosis virus plus some unknown factor favored the male. Results of the daily emergence of this sample are shown in figure 15.

Another random sample was taken near Dos Palos, California, in August. There was no evidence of virus disease, and adults had been emerging for a number of days. The sample consisted of 308 pupae from which 149 males and 159 females emerged. This approaches a 1:1 sex ratio; however, since

the males emerge first, this sample favored the female. If the sample had been taken earlier, a normal, unbalanced sex ratio favoring the male would probably have been obtained.

An early cutting may also favor the male over the female. If a field is cut before most of the pupae have emerged, the female pupae will be killed in larger numbers than the male since the females require more time for development. This in turn will reduce population oviposition by a reduction of females.

TABLE 9

SEX RATIOS FROM FIELD-COLLECTED PUPAE OF *COLIAS PHILODICE EURYTHEME*

Location of field	Month	No. of pupae	Sex ratio (male : female)	Comments
Dos Palos	September	37	3.0 : 1	Females apparently destroyed by polyhedrosis virus.
Westley	August	362	1.4 : 1	Females eliminated by some unknown factor.
Westley	September	467	1.5 : 1	Females destroyed by polyhedrosis virus plus unknown factors.
Dos Palos	August	308	0.9 : 1	Balanced sex ratio obtained because of male emergence prior to sampling.

Age Structure and Dispersal

In uncultivated areas, populations are usually composed of all developmental stages, so that there may be no definite peak of oviposition. On the other hand, in commercial alfalfa-growing areas, where climate generally controls the cutting cycle which, in turn, determines the *Colias* broods, a given population is more often composed of a specific developmental stage. These even broods usually mature and emerge as adults shortly before cutting. The females reach maximum oviposition a few days after emergence, then oviposition gradually decreases. Thus, there is generally a peak oviposition period after each cutting cycle in an alfalfa-producing area.

The age and movements of the females in a population often determine the density of the ensuing larval population (Smith, Bryan, and Allen, 1949). Upon emerging, the females mate at least once in the field from which they emerge, and then disperse more or less at random until a favorable oviposition site is found. There is a tendency for the female to orient against the wind and to be attracted to low, green vegetation, but the movement is not deliberately made to a favorable oviposition site (Smith, Bryan, and Allen, 1949). If oviposition-stage fields (alfalfa fields recently cut and just beginning regrowth) are near an emergence field, many of the first emerging females will concentrate in those fields.

Little food is available for adults in oviposition-stage fields, and the females may return to the emergence field where food is plentiful in the form of blooming alfalfa flowers. On the other hand, the females may dis-

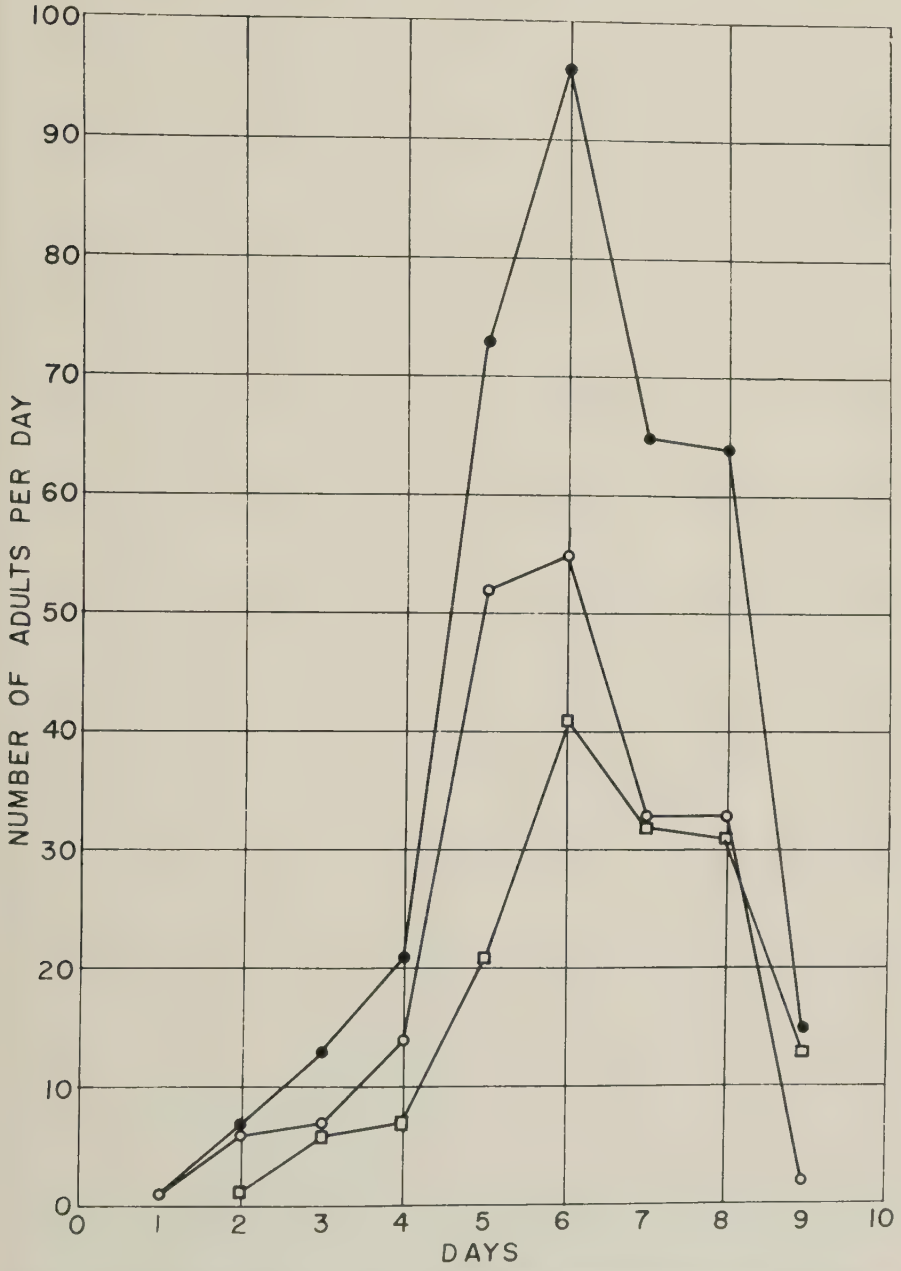

Fig. 14. Daily emergence of male and female *Colias philodice eurytheme* at 20° C. Open circles indicate male emergence; open squares, female emergence; closed circles, combined male and female emergence.

perse to other fields in search of food. If the emergence field is sampled at such a time, it will be seen to be occupied by two age groups of females, those recently eclosed and those that have returned to feed and perhaps to mate a second time. Correspondingly, young females replenish the populations in the oviposition fields. Thus, the oviposition fields will contain a population that is composed of young and old adults. Correspondingly, some of the old females taking nectar in the emergence field will migrate to other oviposition fields.

This type of population movement continues until the pupae in the emergence field have all eclosed; then adult populations gradually decline. Samples of females taken from emergence or oviposition fields early in the cycle

TABLE 10

COMPARISON OF MATURE EGGS PER FEMALE, NUMBER OF SUCCESSFUL MATINGS, AND SIZE OF FAT BODY IN YOUNG AND OLDER FEMALES IN TWO TYPES OF ALFALFA FIELDS

Stage of field	Month	Age group	Number of females	Mean number of mature eggs per female	Mean number of spermatophores per female	Mean of fat body rating*
Emergence	September	Young	16	10	1.0	4
		Old	10	22	1.4	1
Emergence	October	Young	11	4	0.9	4
		Old	9	64	1.4	2
Emergence	October	Young	18	4	1.0	4
		Old	7	51	1.4	2
Oviposition	October	Young	14	2	1.1	4
		Old	9	28	1.7	2

* 4 = highest rating.

yield young females. If a sample is taken in the middle of the population movement cycle, females of all ages will be found, while a sample taken late in the cycle will be composed of old females.

Samples of females taken from emergence and oviposition-stage fields demonstrate this general pattern of female activity. Females were brought into the laboratory and dissected. The age, number of matings, fat body content, and condition of the ovaries were recorded (table 10).

The first sample (26 females) was taken from an emergence field near Westley, California, in the middle of September. Sixteen females were very young, and had mated only once. Six of these contained mature eggs, to give an average of 10 eggs per female for all 16. Their fat body rating was 3.9 (4.0 equals highest rating). Four of the 10 older females had mated twice, while six had mated but once. All 10 contained mature eggs. Three of the 10 were very old, and contained only a few mature eggs. The 10 older females had a fat body rating of 1.4.

A second sample (20 females) was taken near Davis, California, from an emergence field during the first part of October. Eleven females were

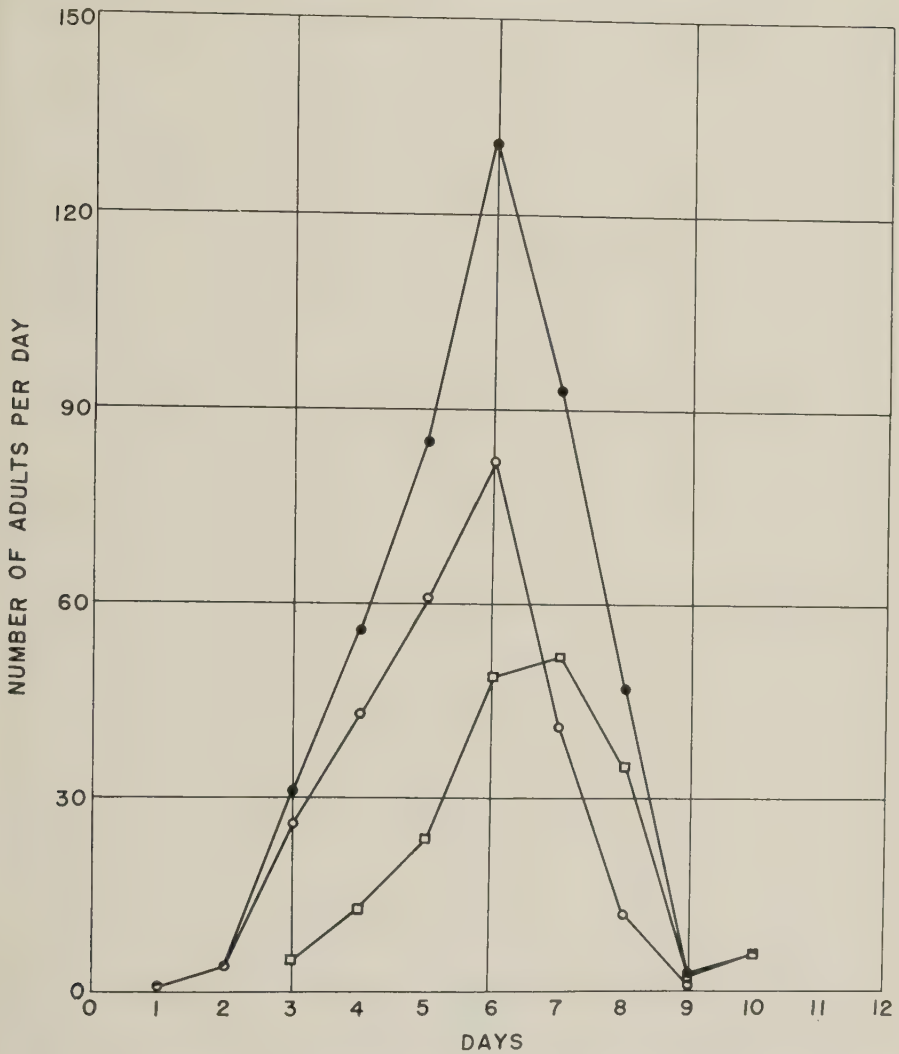

Fig. 15. Daily emergence of male and female *Colias philodice eurytheme* at 20° C. Open circles indicate male emergence; squares, female emergence; closed circles, total male and female emergence.

young. Ten of these had mated once, and the other had not mated. Three of the 10 contained a few mature eggs. The fat body rating for this group was 4.0. Each of the nine remaining older females contained mature eggs (average, 64.4). Their fat body rating was 2.0. Four of the nine had mated twice, while five had mated once.

The third sample (25 females) was taken from an emergence field near Firebaugh, California, in late October. This field was a half mile from the oviposition-stage field discussed below. Eighteen of the females were very

young, and each had mated once. Only two contained mature eggs, 32 and 48, respectively. Their fat body rating was 4.0. Three of the remaining seven old females had mated twice, while the remaining four had mated once. All seven contained mature eggs, with an average of 50.7 eggs per female. Their fat body rating was 2.4.

A fourth sample (23 females) was taken near Firebaugh, California, from an oviposition-stage field in the last part of October. This field was a half mile from the emergence field mentioned above. Thirteen of the 14 young females in the sample had mated once; the remaining female had mated twice. The spermatophore from the first mating was very small; the second was medium-sized. This female did not contain mature eggs. Of the 14 young females, one contained 28 mature eggs, the remaining 13 had eggs less than one-half the mature size. This young group had a fat body rating of 4.0. One of the nine old females had mated three times; four had mated twice; and four had mated once, for an average of 1.7 matings. All nine contained mature eggs (average, 27.7). Their fat body rating was 1.7.

Of the four samples, totaling 94 females, 17 females had mated more than once. One of these was young, with no mature eggs, and a fat body rating of 4.0. The spermatophore from the first mating was very small. Another had a fat body rating of 3.0, while the remaining 15 had either a medium amount, or little or no fat body. These 15 females had an average fat body rating of 1.9, further indicating that multiple matings occur in older females. The females that had been mated only once had an average fat body rating of 3.5. By far the largest number of this group were freshly emerged or young females. However, some of those that were mated but once had little or no fat body, showing that not all females multiply mate.

SUMMARY

The reproductive system of *Colias philodice eurytheme* does not differ greatly from those of other Lepidoptera with two genital apertures. The mating of this insect usually occurs a few hours after emergence, although during cool weather, or in areas of very low population density, mating may not occur on the first or even on the second day after emergence. Prior to mating there appears to be a definite precopulatory pattern of activity. A successful mating requires the passage of a spermatophore. This structure, which is formed by the glandular secretions of the male, is hard, spherical, slightly flattened on the top, and with a long, cone-shaped neck at one end. In nature, all fully hardened females of normal appearance were found to be successfully mated at least once; some were found that had been mated four times.

Prior to oviposition, this species must pass through a brief preoviposition period. The duration of this period is largely controlled by temperature and available food, both of which greatly affect oögenesis. If the female is mated soon after emergence, the spermatozoa reach the spermatheca before the first eggs mature. Egg maturation is greatly decreased unless some carbohydrate is available in the diet. At very low temperatures the female often dies before many eggs mature.

Studies on the environment show that temperature is generally more critical than humidity, light intensity, or food in controlling the oviposition

rates of *Colias*. The optimum body temperature for high rate of oviposition appears to be 32° C, although body temperatures over a range of 28° to 36° C are nearly as satisfactory. Above and below this range, oviposition decreases. The upper and lower thermal limits for oviposition are 46° C and 21° C body temperature, respectively. The optimum humidity for a high oviposition rate ranges from 26 to 45 per cent. As the humidity is increased or decreased beyond that range, oviposition decreases. At the higher and lower temperatures, any effect that humidity may have on oviposition is masked by the effects of temperature. The optimum temperature and humidity for high oviposition rates in the laboratory agree with the optimum temperature and humidity under field conditions. Light intensity, above a certain minimum, is perhaps more significant in its heating effect than it is as an independent factor controlling oviposition. Food is more important in its effect on oögenesis than on oviposition, and some sugar is required in the diet for high egg maturation.

It was found that a female may lay as many as 1,172 eggs, but the average is about 715. Phenotypically yellow females appear to be more prolific than white females. The mean longevity of this group, which was tested for the reproductive potential, was 21.8 days. There is a gradual decrease in fat body size as the number of eggs oviposited increases. The sex ratio of *C. philodice eurytheme* tends to favor the male. This appears due to the fact that the developmental rate of the female is slower in the larval stages than is that of the male. The female is therefore subjected to adverse conditions in the larval stage over a longer period of time. The polyhedrosis virus disease can be significant in changing the sex ratio. In other cases, unknown factors appear to reduce the more slowly developing female. Young, middle-aged, and old females may be found in emergence and oviposition fields. In order to determine the reproductive potential of a population, the age, fat body content, and the number of matings of various classes of the population must be determined by dissection.

ACKNOWLEDGMENTS

The authors wish to express their sincere appreciation to William W. Allen, Douglas E. Bryan, and Thomas F. Leigh, who assisted us with some phases of both the field and laboratory investigations. Many valuable suggestions and criticisms have been offered by our colleagues P. F. Bonhag, K. S. Hagen, B. A. Jones, and E. G. Linsley, to whom we are indeed grateful.

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